SPECIAL COLLECTOR'S EDITION

SCIENTIFIC AMERICAN

DETECTING EARTHQUAKES IN THE SKY

BABIES WITHOUT SEX

WILD IDEAS IN SCIENCE

PULLING WATER FROM THIN AIR

21

THE SEWAGE CURE FOR DIABETES

GROWING HUMAN BRAINS IN A PETRI DISH

AMAZING INNOVATIONS THAT JUST MIGHT SAVE THE PLANET AND US

SUMMER 2019

© 2019 Scientific American

Expertise. Insights. Illumination.

Discover world-changing science. Get 12 issues of *Scientific American* in print and explore our digital archive back to 1845, including articles by more than 150 Nobel Prize winners.

sciam.com/print&archive

Download on the App Store

GET IT ON Google Play

SPECIAL ISSUE : SEX AND GENDER
GENDER MYTHS DEBUNKED
SPEAKING IN WHISTLES
WHAT'S WRONG WITH COSMIC INFLATION?
WORKOUTS AND WEIGHT LOSS
Learn the surprising evolutionary reason why exercise alon won't shed pounds—and what to do about it
PLUS
PREVENTING THREATS TO CROPS
Airborne microbes' critical role
WHAT IS A KILOGRAM?
Science seeks new ways to ensure accuracy
MEDICAL SCANS
A shortage of rare atoms imperils disease imaging
SCIENTIFIC AMERICAN
AUTOMOBILE NUME
T. REX reexamined
SCIENTIFIC AMERICAN
September 1999

Scientific American is a registered trademark of Springer Nature America, Inc. Google Play and the Google Play logo are trademarks of Google LLC. Apple, the Apple logo, iPhone, and iPad are trademarks of Apple Inc., registered in the U.S. and other countries and regions. App Store is a service mark of Apple Inc.

SCIENTIFIC AMERICAN®
ESTABLISHED 1845

Wild Ideas in Science is published by the staff of Scientific American, with project management by:

Editor in Chief and Senior Vice President: **Mariette DiChristina**

Managing Editor: **Curtis Brainard**

Senior Editor, Collections: **Andrea Gawrylewski**

Creative Director: **Michael Mrak**

Issue Designer: **Lawrence R. Gendron**

Photography Editor: **Monica Bradley**

Assistant Photo Editor: **Liz Tormes**

Copy Director: **Maria-Christina Keller**

Senior Copy Editor: **Daniel C. Schlenoff**

Copy Editor: **Aaron Shattuck**

Managing Production Editor: **Richard Hunt**

Prepress and Quality Manager: **Silvia De Santis**

Editorial Administrator: **Ericka Skirpan**

Senior Secretary: **Maya Harty**

President: **Dean Sanderson**

Executive Vice President: **Michael Florek**

Vice President, Commercial: **Andrew Douglas**

Publisher and Vice President: **Jeremy A. Abbate**

Associate Vice President, Business Development: **Diane McGarvey**

Head, Marketing and Product Management: **Richard Zinken**

Marketing Director, Institutional Partnership and Customer Development: **Jessica Cole**

Programmatic Product Manager: **Zoya Lysak**

E-mail Marketing Manager: **Chris Monello**

Marketing and Customer Service Coordinator: **Christine Kaelin**

Head, Communications, USA: **Rachel Scheer**

Director, Integrated Media: **Jay Berfas**

Director, Integrated Media: **Matt Bondlow**

Custom Publishing Editor: **Lisa Pallatroni**

Rights and Permissions Manager: **Felicia Ruocco**

Senior Administrator, Executive Services: **May Jung**

Production Controller: **Madelyn Keyes-Milch**

Advertising Production Controller: **Dan Chen**

GETTY IMAGES

Meat cultured from stem cells in laboratory conditions

FROM THE EDITOR

Call of the Wild

In 1956 psychologist J. P. Guilford coined the term "divergent thinking" to describe a thought process that generates out-of-the-box ideas and innovations. It's used by individuals who create surprising solutions to problems that no one else considers. This kind of thinking fills the following pages—the most far-out ideas to tackle some of our biggest challenges, from climate change and water availability to species extinction and debilitating human disease. These atypical, and sometimes controversial, scientific endeavors employ unusual approaches that seem far-fetched but may just save the day.

Take some of these whiz-bang solutions for sustainable living. Rebuilt swamps and waterways in the middle of so-called sponge cities can absorb excess rainfall and clean urban water caches (*page 4*). Solar-powered panels can pull water directly out of the air for human use (*page 18*), and technology that mimics photosynthesis in a leaf can produce fuels to power cars and homes (*page 10*). Even more strange, perhaps, is that we may need to look to the sky to spot early warning signs of impending earthquakes (*page 20*).

As humans alter the planet, other forms of life have to keep up, but some out-of-the-ordinary experiments may help. Several research groups are developing tools to accelerate gene flow in plants and animals, to create varietals of trees (*page 30*) and corals (*page 36*) that can adapt to their changing environments. Genetic manipulation could also finally mean control over invasive species in the Galápagos that are wreaking havoc on native flora and fauna (*page 46*)—or we could just eat them into submission (*page 56*).

Unconventional lines of inquiry are also at the core of the latest advances in human health. Researchers are using a CRISPR recording device programmed into bacterial cells to track everything from infections in the body to elevated pollutant levels in water sources (*page 77*). And what if electrical stimulations administered directly to the vagus nerve could treat a handful of chronic conditions better than any drug (*page 76*)? An engineered and fluorescent version of the rabies virus is lighting up neuropathways for study (*page 110*), while lab-grown brains are revealing the origin of disorders from autism to Alzheimer's disease (*page 90*).

Human ingenuity keeps pushing innovation forward, far beyond what was imagined even a few years ago. The challenges we face today feed the machine of creative science on which hangs the sign: Wild Ideas Welcome.

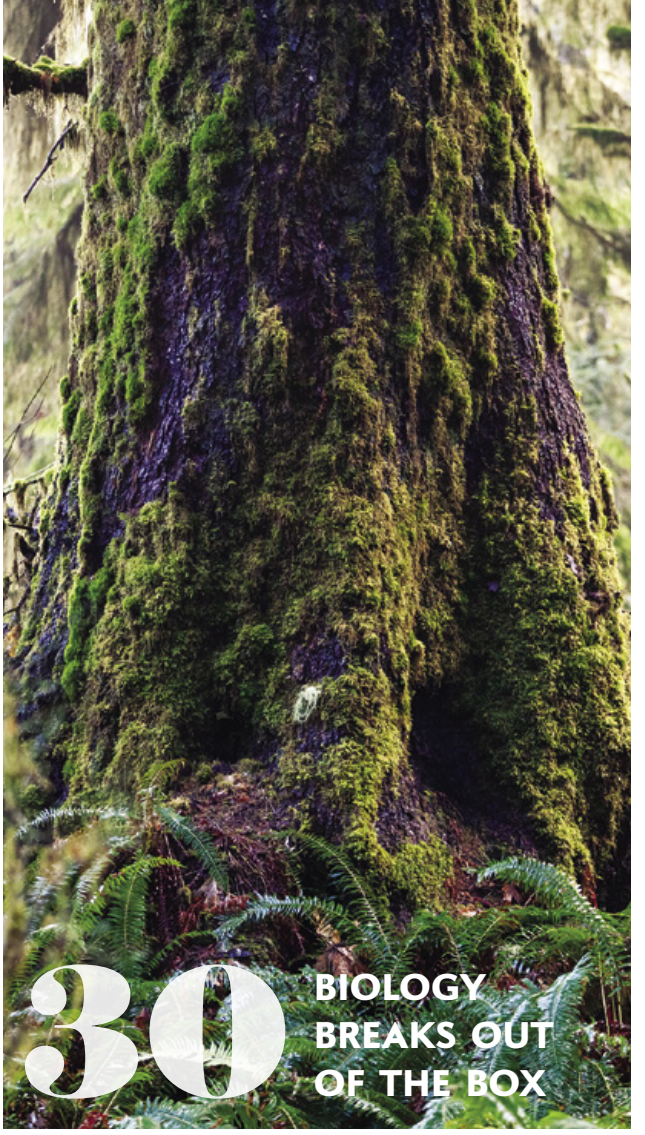
Andrea Gawrylewski
Senior Editor, Collections
editors@sciam.com

© 2019 Scientific American

SPECIAL EDITION

SCIENTIFIC AMERICAN

Volume 28, Number 3, Summer 2019



4 Sponge Cities
Restoring natural water flows—by adding back swamps and streams in cities—can lessen the impacts of floods and droughts. *By Erica Gies*

10 Fuel from an Artificial Leaf
Technology that mimics photosynthesis converts carbon dioxide to fuels in a sustainable way. *By Javier Garcia Martinez*

12 High-Flying Microbes
Aerial drones and chaos theory help researchers explore the many ways that microorganisms spread havoc around the world. *By David Schmale and Shane Ross*

18 Water Made by the Sun
Technologies that pull moisture from the air are now solar-powered. *By Donna J. Nelson and Jeffrey Carbeck*

20 Earthquakes in the Sky
The best early warnings of a big disaster may appear 180 miles above the ground, according to a controversial new theory. *By Erik Vance*

26 The Universal Problem of Climate Change
Solutions to Earth's troubles may come from astrobiology. *By Lee Billings*

30 Forests on the March
Trees can't walk to a better place as climate worsens. So scientists are relocating helpful genes instead. *By Hillary Rosner*

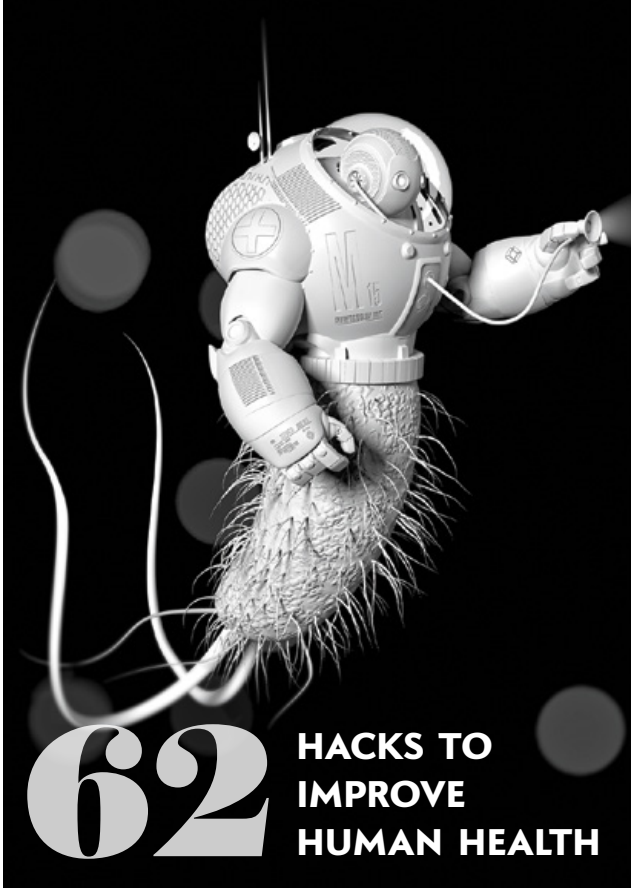
36 Can We Save the Corals?
Scientists are urgently transplanting, fertilizing and enhancing corals to help them adapt to warmer oceans, but rebuilding entire reefs will be daunting. *By Rebecca Albright*

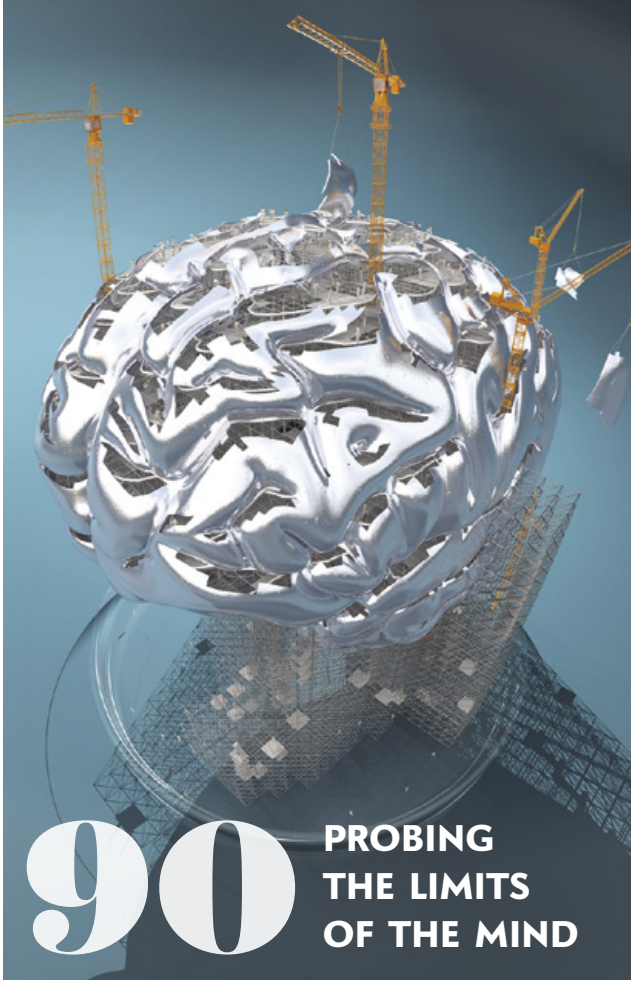
44 Meat Grown from Stem Cells
Beef for dinner—without killing animals or the environment. *By G. Owen Schaefer*

46 Could Genetic Engineering Save the Galápagos?
In the Galápagos, invasive species are driving native animals to extinction. Some conservationists are asking whether genetic manipulation is the solution. *By Stephen S. Hall*

56 Can We Eat Invasive Species into Submission?
The tale of a giant Amazon fish reveals the promise and peril of "invasivorism." *By Michael Snyder*

© 2019 Scientific American





62 Transformers
By reprogramming DNA inside harmful microbes, biologists are turning them into patient-saving drugs. *By Michael Waldholz*
Also: **Unnatural Responsibilities** *By Kevin M. Esvelt*

70 The War on Slime
Biofilms—3-D mats of bacteria—kill as many people as cancer does and fight off antibiotics. Scientists are using their own weapons against them. *By Karin Sauer*

76 Electroceuticals
Nerve-stimulating therapies could replace drugs for many chronic conditions. *By Geoffrey Ling and Corinna E. Lathan*

77 Bacterial Tape Recorder
Researchers program bacteria to record cellular memories into tiny recording devices. *By Yasemin Saplakoglu*

78 The Means of Reproduction
Could scientists one day use blood and skin cells to replace sperm and eggs? *By Karen Weintraub*

84 Vanquishing Diabetes
Cleaner environments in the industrial world have led to an increase in the incidence of type 1 diabetes. This history shows the way to a novel vaccine. *By Kristen M. Drescher and Steven Tracy*

90 Lab-Built Brains
Scientists copy nature's most complex organ in the hope of solving the mysteries of brain disorders, from autism to Alzheimer's. *By Juergen A. Knoblich*

96 Out of the Silence
After false starts, researchers are making progress toward treating deafness with gene therapy. *By Dina Fine Maron*

104 Sleep Learning Gets Real
Experimental techniques demonstrate how to strengthen memories when our brains are off-line. *By Ken A. Paller and Delphine Oudiette*

110 Rabies on the Brain
Using engineered forms of the rabies virus, neuroscientists can map brain circuits with unprecedented precision. *By Andrew J. Murray*

DEPARTMENTS

1 FROM THE EDITOR: Call of the Wild

116 END NOTE: Googling Heroin
Internet searches offer a novel way to predict overdose deaths. *By Rod McCullom*

Articles in this special issue are updated or adapted from previous issues of *Scientific American* and *Nature* and from ScientificAmerican.com.
Copyright © 2019 Scientific American, a division of Springer Nature America, Inc. All rights reserved. Scientific American Special (ISSN 1936-1513), Volume 28, Number 3, Summer 2019, published by Scientific American, a division of Springer Nature America, Inc., 1 New York Plaza, Suite 4600, New York, N.Y. 10004-1562. Canadian BN No. 127387652RT; TVQ1218059275 TQ0001. To purchase additional quantities: U.S., $13.95 each; elsewhere, $17.95 each. Send payment to Scientific American Back Issues, 1 New York Plaza, Suite 4600, New York, N.Y. 10004-1562. **Inquiries: fax 212-355-0408 or telephone 212-451-8415. Printed in U.S.A.**

© 2019 Scientific American

SPONGE

Restoring natural water flows in cities can lessen the impacts of floods

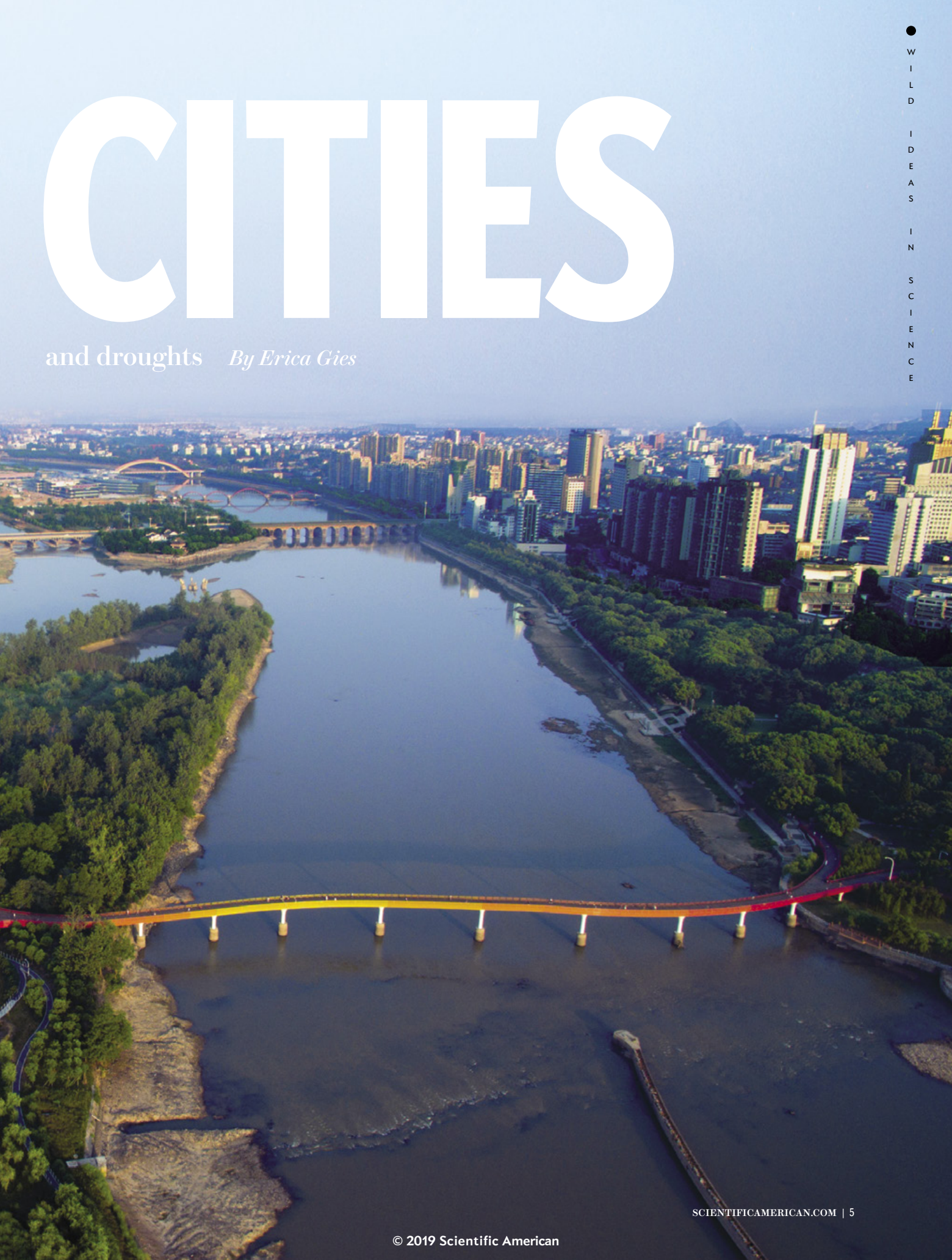
YANWEIZHOU PARK (*center*) absorbed high river water during a heavy monsoon, protecting Jinhua, China, from flooding.

© 2019 Scientific American

CITIES

and droughts

By Erica Gies



© 2019 Scientific American

ELEVEN INCHES OF RAIN CHUCKED down on Beijing on July 21, 2012, flooding roads and filling underpasses. Landscape architect Yu Kongjian barely made it home from work. "I was lucky," he says. "I saw many people abandon their cars." As the deluge continued, the city descended into chaos. Beijing's largest storm in more than 60 years killed 79 people; most of them drowned in their vehicles or were sucked into underground drains. Damages reached nearly $2 billion.

To Yu, co-founder of the internationally acclaimed landscape architecture firm Turenscape, the disaster was avoidable, a consequence of heedless development. He had warned the city government several years earlier, after he led a research team mapping the metropolis's "ecological security pattern," identifying land with high flood risk that should be left undeveloped and used to manage stormwater. "The 2012 flood gave us the lesson that ecological security patterns are a life-and-death issue," he says.

A similar story has played out across China. Sixty-two percent of its cities flooded between 2011 and 2014 alone, imposing $100 billion in economic losses, according to the Chinese Ministry of Housing and Urban-Rural Development. The floods are partly the result of stronger storms fueled by climate change. But the harm is mostly self-inflicted: intensive urbanization over the past 30 years has gobbled up wetlands, felled forests, paved over farms and grasslands, and channeled rivers in concrete straitjackets, leaving stormwater that once filtered into the ground nowhere to go but up.

Urban sprawl is exacerbating water scarcity in China, too. Buildings, streets and parking lots block rain from recharging aquifers. Instead drains and pipes funnel it away—lunacy in a place with water shortages, Yu thinks. Like other cities in China's north, Beijing is pretty dry outside of the summer monsoon season. For decades it has pumped groundwater to supply its growing population and consumption. The city is lowering the water table about a meter a year, causing the ground to sink as well.

Cities worldwide share similar problems because of development and the attempt to control water with "gray" infrastructure—concrete dams, levees, stormwater tanks, pipes and walled-in rivers whose floodplains are covered with buildings. Experts are recognizing that by breaking the natural water cycle, municipalities are raising the likelihood and severity of flooding, causing disasters from Houston to Chennai, India.

Yu is at the forefront of a global movement of urban planners, water managers, ecologists and engineers who are trying to restore natural water cycles. The work is a kind of un-engineering: giving water space to expand and contract to lessen flooding and slowing it down so it can soak into the ground, preventing shortages later. Practitioners preserve or restore floodplains and wetlands, unearth buried creeks, and create bioswales, retention ponds, sunken parks and permeable parking lots. Unlike hardscapes, green infrastructure can also clean water and re-create habitat for wildlife. And it gives urbanites access to nature, an amenity increasingly recognized as a pillar of mental health.

Local landscape design projects are popping up everywhere. But Yu and other leading practitioners are looking to manage water at a grander scale: an entire city, an entire watershed. Known as green infrastructure in Europe, low-impact development in the U.S. and sponge cities in China, these approaches mimic nature as much as possible, says Tony Wong, an engineer and chief executive of the Cooperative Research Center for Water Sensitive Cities, based in Melbourne, Australia. The goal is to create water infrastructure that "functions as a living organism," he says.

The sponge cities movement is gaining momentum. In March 2018 the United Nations released a report called *Nature-Based Solutions for Water* that supports the approach. The U.S. Army Corps of Engineers, infamous for its muscular engineering of rivers and wetlands, now has an Engineering with Nature initiative. After centuries of dikes to enclose rivers and low-lying land, the Dutch are onboard, too. A near disaster in 1995, when water rose in the Rijn, Maas and Waal rivers, forcing 250,000 people to evacuate, prompted the government to create a countrywide program called Room for the River. Instead of only building bigger dams and dikes, Dutch officials increased the capacity of river deltas by asking farmers to agree to move or to let their land flood as necessary.

China, with its rapid growth and centralized government, is pursuing sponge cities on a scale difficult for most countries to even consider. The ambition is impressive. Yet Yu is finding that he still has to overcome a tendency among planners toward one-size-fits-all approaches, which could be disastrous because each location has unique hydrological systems and needs. He is also confronting a penchant in his country for stronger dams, bigger pipes and larger storage tanks, which symbolize power and progress in modern China.

MR. SPONGE CITY

ON A SPRING DAY in Beijing with a "very high" air pollution rating, I visited Yu at Turenscape's headquarters in the city's

IN BRIEF

Floods and droughts are crippling many urban areas. Concrete river channels, stormwater tanks and pipes are not keeping up.

An urban-planning approach called sponge cities can more effectively lessen floods, save water for dry spells and reduce water pollution.

Yu Kongjian is leading the way as China reengineers old cities and designs new ones to embrace rather than fight natural water flows.

PRECEDING PAGES: TURENSCAPE

© 2019 Scientific American

KABAN LAKES in Kazan, Russia, were polluted and flood-prone. Now their redesigned banks absorb and clean urban runoff.

Haidian District. A slim, intense man in his 50s, with sharp eyes and just a bit of gray at the temples, Yu traces his passion to the agricultural commune where he grew up in Zhejiang province southwest of Shanghai. There he observed the Chinese "peasant wisdom" for managing water, practiced for thousands of years. Farmers maintained little ponds and berms to help rainfall infiltrate the ground, storing it for a dry day. The creek next to his village swelled during certain seasons, which no one saw as a threat. "If you have wise ways to deal with flooding, water can be friendly," he says.

Since starting Turenscape in 1998 with his wife and a friend, Yu has built the award-winning company into a landscape architecture empire with 600 employees. The company has more than 640 projects built or underway in 250 Chinese cities and 10 other countries, including a redesign of the Kaban Lakes system in Kazan, Russia. Yu is dean of the school of landscape architecture at Peking University and has taught periodically at Harvard University.

For years while Yu was building his firm's portfolio, many Chinese derided his farm-based ideas as backward. He says that some even called him an American spy—a nod to his doctorate from Harvard's Graduate School of Design and his opposition to those big dams. But in recent years sentiment has begun to shift. Various groups in China are building green infrastructure projects, often in partnership with Americans, Australians and Europeans. Yu's influence has been growing in parallel. He lectures regularly at the Ministry of Housing and Urban-Rural Development, and his 2003 book about his practices, *The Road to Urban Landscape: A Dialogue with the Mayors,* has been printed multiple times in China. Dignitaries such as the Mexican ambassador to China, who is hoping he can solve Mexico City's water problems, ask for his input.

The 2012 Beijing flood was a turning point. Soon afterward, a Turenscape stormwater project in Harbin won a top U.S. design prize. China Central Television broadcast a long, high-profile interview with Yu. A government minister told him afterward that Xi Jinping, who would soon become president, had seen it. About a year later the president stood before China's central urbanization conference and announced his Sponge City initiative, elevating the idea from struggling concept to national goal.

In 2015 the government initiated 16 demonstration projects, each covering at least 10 square kilometers. Today there are 30. The objectives include reducing urban flooding, retaining water for future use, cleaning up polluted water bodies and improving natural ecosystems. By 2020 each project is supposed to retain 70 to 90 percent of the site's average annual rainfall. Premier Li Keqiang said in his 2017 government work report that sponge city construction will continue to expand.

A RIVER RECONSTRUCTED

A WEEK AFTER MEETING YU, I visited one of Turenscape's latest projects, Yongxing River Park, in a Beijing exurb called Daxing. "Before" satellite pictures from three years ago show open land surrounding the river, already straightened and confined by steep concrete walls. "Now" pictures are chockablock with buildings. Showing me the park are two of Yu's employees, Geng Ran, who goes by Katie when talking to English speakers, and Zhang Mengyue, aka Sophie.

The government recognizes that development reduces rain infiltration, Zhang says, so it invited Turenscape to design a park that would enlarge the riverbed to hold more water. The project was nearly completed when I saw it in early April 2018. About four kilometers long and perhaps two city blocks wide, the park follows the river. We stand on a large berm that divides the riverbed into two channels. The river flows on our right; on our left the channel has big dirt holes of varying depths. During the dry season, the holes will be filled with partially cleaned effluent from a sewage treatment plant. Wetland plants in the pools will further clean the water, Geng says, and filter some of it into aqui-

TURENSCAPE

© 2019 Scientific American

fers. During the monsoon, the channel will be reserved for floodwaters, and the effluent will be treated industrially.

"We say you can't stop the weight of the river," Geng says, "so that's why we enhance this river." Turenscape's plan involved removing concrete along the river and excavating soil to widen the bed. That dirt was then molded into the big berm. Thousands of small sedges planted in closely set rows now dot those riverbanks, holding the earth in place. They remind me of Georges Seurat's pointillism. Turenscape projects use native plants because they "are adapted to the local environment," Yu says, "and need no supplemental water."

Earlier installations are already demonstrating their efficacy. Yanweizhou Park in Jinhua, near where Yu grew up, absorbed a 100-year flood, protecting the city. Shanghai's 14-hectare (34.6-acre) Houtan Park cleans up to 2,400 cubic meters (634,000 gallons) of polluted river water daily, improving the water's quality from grade V (unsuitable for human contact) to grade II (suitable for landscape irrigation) using only biological processes.

Projects like these are most effective when they connect to other green infrastructure throughout the watershed, enabling water to flow in an approximation of its natural path. Across China, whole new cities being built from scratch show what is possible. Turenscape has completed part of the ambitious Wulijie Eco-City in Hubei province. Wulijie's design preserves the natural wetlands for catching and cleaning stormwater on-site. This approach reduced construction costs for underground drainage pipes and conserved habitat for wildlife and vegetation. Buildings have roof gardens and living walls, and pedestrian and bike paths thread through the green space, all of which should enhance quality of life for residents.

BEYOND CITIES

IT'S NOT EASY to make space for water in an environment that has already been built, however. Architects have to shoehorn tiny projects into existing infrastructure. In Houston, for example, developers often limit themselves to building bioswales in new apartment complexes. In San Francisco, workers have been jackhammering bits of sidewalk and roadway medians to make room for plantings.

Hence the appeal of derelict industrial sites ripe for dramatic reclamation. Turenscape oversaw the first phase of such a project in the 1,000-year-old city of Kazan, which surrounds three oxbow lakes on the Volga River. During the Soviet period, pollution had killed nearly all life in the lakes. Moreover, the city was prone to flooding because of the way dams were built. When waters would rise, the city's seven pumping stations could not keep up.

Turenscape's design, which is partially complete, calls for reclaiming 11 square kilometers of land for floodwater along the river and its tributaries. There the city is building linear parks, promenades and bioswales that slow, absorb and clean urban runoff before releasing it into the lakes. Walking and biking routes give people access to the riparian zone and support human-powered transportation throughout the city.

Such extensive redesigns are encouraging Yu to dream beyond sponge cities to what he calls "Sponge Land." His inspiration is to take care of the national landscape. "Water is a system," he says, bigger than cities.

Inspired in part by American landscape designer Warren Manning, who created "A National Plan" for the U.S. a century ago, Yu is working on a landscape master plan for all of China. "That is an incredible vision," says Niall Kirkwood, a professor of landscape architecture and technology at the Harvard Graduate School of Design, who has known Yu for many years. "No one thinks at that scale and with that political savviness."

1

2

Yu's office walls display maps of China that document elevation, watersheds and flood paths, as well as biodiversity, desertification, ecological security, soil erosion and cultural heritage. For his big plan, Yu can use them along with geographic information system (GIS) and satellite imagery to track China's landscape changes as urbanization spreads, as estuaries and deltas silt up, as water starts to move differently across landscapes and cityscapes. He can isolate priority areas where projects will have the biggest impact. Kirkwood says this is like applying acupuncture to the human body. Yu "has the understanding that doing a piece of work in one area will have an effect in another area," he says. Compared with most landscape architects, Kirkwood says, Yu is "thinking much more holistically."

CUSTOM SOLUTIONS NEEDED

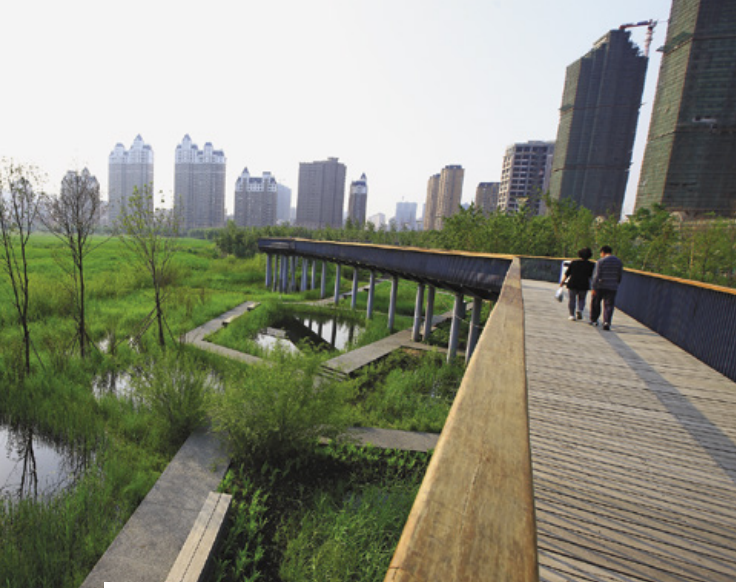
YU'S HOUSE, a duplex of side-by-side apartments where he and his sister live, is a personal testament to his ideas. Between the two apartments is a living wall Yu built of porous limestone. Water captured from the roof dribbles down its face, from which maidenhair ferns and philodendrons sprout. The green wall cools the two homes enough that they do not need air-conditioning, he says, although he concedes that it gets a bit warm in summer.

Decks off the bedrooms are watered with roof-caught rain, stored in tanks under plant beds. Yu's deck smells great, emanating whiffs of rosemary, lemongrass and Chinese chrysanthemums. It even has a tiny creek in which goldfish swim. On the other side of the wall, his sister's deck has terraced beds replete with lettuce and chard. "We collect 52 cubic meters of stormwater [annually], and I grow 32 kilograms of vegetables," Yu says proudly.

The ideas engineered into Yu's house are widely applicable to buildings, but each sponge city design must be unique, fac-

TURENSCAPE

© 2019 Scientific American



YU KONGJIAN (*1*), framed by the "living wall" inside his home, has championed China's sponge city projects, including a stormwater park in Harbin (*2*).

toring in the site's local climate, soil and hydrogeology. "Every patient needs a different solution," as Yu says. There is a danger that, in their haste, Chinese planners will ignore this fact. If they do, the broad ambition for sponge cities may falter, says Chris Zevenbergen, an expert in urban flood-risk management at the IHE Delft Institute for Water Education in the Netherlands and a visiting professor in Nanjing and Chengdu. The tendency to hurriedly pursue cookie-cutter solutions in erecting cities over the past 20 years did not allow builders time to understand imperfections in design and adjust. That is why so many cities have ongoing problems with floods, he says. Copycat implementation of sponge cities could lead to similar problems. Xi's program has strict deadlines, which may not provide time to monitor performance and adjust if necessary, Zevenbergen says.

A paper written by Chinese government research institutes in 2017 expressed similar concerns. To provide guidance, the government has formed a committee that is composed of civil engineers, economists and landscape architects, including Yu.

The sponge city vision faces other challenges. It will take private investment to fully implement the national plan. But Yu worries that companies might find pipes or dikes—things they can charge for—more attractive than sponge cities' embrace of natural systems.

The stakes for the grand vision go beyond tempering floods and drought. Xi also wants sponge cities to deal with another big water issue China faces: pollution. Nutrients, heavy metals, pesticides and microplastics taint surface waters in China, according to Randy Dahlgren, a scientist at the University of California, Davis, who specializes in soil and water chemistry and has worked in Zhejiang province. "If they can get this water to infiltrate into the ground, a huge number of these potential contaminants will be retained within the wetlands systems, buffers, detention basins and bioswales," he says.

Wetlands cannot just be built and forgotten, however. Phosphorus, heavy metals and some nitrogen can accumulate in the plants and return to the soil when those plants die. "You really need to be harvesting those plants," Dahlgren says. They can be made into biomass fuels and incinerated, although some pollutants such as metals gather in the ash, which must be disposed of. "It does take active management of wetlands to make them an effective sink for a lot of pollutants," he says. Planners should also be cautious about "trading a surface-water pollution issue for a groundwater pollution issue," where impurities could persist for tens of years to several centuries.

If the planners get it right, though, the payoff could be huge. Sponge city techniques are already reducing pollution in places such as Philadelphia. Like many U.S. cities, its stormwater runs through sewage-treatment plants, which overflow in big storms, pushing untreated sewage into rivers. With its Green City, Clean Waters initiative, the city is reclaiming land along the banks of local creeks and rivers to absorb excessive rainfall and building parks that can flood when necessary. Philadelphia also gives incentives to landowners for creating rain gardens, green roofs, urban farms and porous pavement. These techniques allow stormwater to percolate into the ground, reducing the volume entering the sewage system. Five years in, the city had "greened" 339 hectares, enough to reduce pollution from sewer overflows by more than 5.7 million cubic meters annually.

STATE OF FLUX

NATURAL WATER-MANAGEMENT SYSTEMS are not static or predictable like gray infrastructure: nature is messy. Water rises and falls. Plants sprout, live and die. Mud is exposed. Although these spaces can be beautiful—perhaps more beautiful than, say, a dam—residents might not always like what they see. For sponge cities to spread, people will have to accept a dynamic environment.

Yu calls this shift "big feet aesthetics," a counterreference to when Chinese considered the bound, tiny feet of aristocratic women to be beautiful because they were useless, a sign that the women were too rich to work. "Now we need to find big feet attractive," he says. "We need to change our aesthetic to find useful green infrastructure beautiful."

Educators will need a change in perspective as well. Despite the national promotion of sponge cities, China's schools are still training engineers using 20th-century principles, Yu says. "We are fighting so hard to try to get people to think in an ecological way." The hubris of believing that people can control water with concrete will be increasingly exposed as more of those kinds of projects fail, unable to buffer the knock-on impacts from rapid population growth, urban sprawl and climate change. Although sponge cities will likely not protect everyone from these challenges, their advocates think their resilience can temper extremes better than the concrete alternatives. Plus the multiple benefits they bring can make the lives of humans and other species healthier and happier. SA

Erica Gies writes about science and the environment from Victoria, B.C., and San Francisco. Her work appears in *Nature*, bioGraphic, the *New York Times*, and elsewhere.

MORE TO EXPLORE

Letters to the Leaders of China: Kongjian Yu and the Future of the Chinese City. Edited by Terreform. Terreform, 2018.

The United Nations World Water Development Report 2018: Nature-Based Solutions for Water. UNESCO, 2018.

scientificamerican.com/magazine/sa

© 2019 Scientific American

Fuel from an Artificial Leaf

Technology that mimics photosynthesis converts carbon dioxide to fuels in a sustainable way

By Javier Garcia Martinez

THE NOTION OF AN ARTIFICIAL LEAF MAKES SO MUCH sense. Leaves, of course, harness energy from the sun to turn carbon dioxide into the carbohydrates that power a plant's cellular activities.

For decades scientists have been working to devise a process similar to photosynthesis to generate a fuel that could be stored for later use. This could solve a major challenge of solar and wind power—providing a way to stow the energy when the sun is not shining and the air is still.

Many, many investigators have contributed over the years to the development of a form of artificial photosynthesis in which sunlight-activated catalysts split water molecules to yield oxygen and hydrogen—the latter being a valuable chemical for a wide range of sustainable technologies. A step closer to actual photosynthesis would be to employ this hydrogen in a reduction reaction that converts CO_2 into hydrocarbons. Like a real leaf, this system would use only CO_2, water and sunlight to produce fuels. The achievement could be revolutionary, enabling creation of a closed system in which carbon dioxide emitted by combustion was transformed back into fuel instead of adding to the greenhouse gases in the atmosphere.

Several researchers are pursuing this goal. One group has demonstrated that it is possible to combine water splitting and CO_2 conversion into fuels in one system with high efficiency. In a June 2016 issue of *Science,* Daniel G. Nocera and Pamela A. Silver, both at Harvard University, and their colleagues reported on an approach to making liquid fuel (specifically fusel alcohols) that far exceeds a natural leaf's conversion of carbon dioxide to carbohydrates. A plant uses just 1 percent of the energy it receives from the sun to make glucose, whereas the artificial system achieved roughly 10 percent efficiency in converting carbon dioxide to fuel, the equivalent of pulling 180 grams of carbon dioxide from the air per kilowatt-hour of electricity generated.

The investigators paired inorganic, solar water-splitting technology (designed to use only biocompatible materials and to avoid creating toxic compounds) with microbes specially engineered to produce fuel, all in a single container. Remarkably, these metabolically engineered bacteria generated a wide variety of fuels and other chemical products even at low CO_2 concentrations. The approach is ready for scaling up to the extent that the catalysts already contain cheap, readily obtainable metals. But investigators still need to greatly increase fuel production. Nocera says the team is working on prototyping the technology and is in partnership discussions with several companies.

Nocera has an even bigger vision for the basic technology. Beyond producing hydrogen- and carbon-rich fuels in a sustainable way, he has demonstrated that equipping the system with a different metabolically altered bacterium can produce nitrogen-based fertilizer right in the soil, an approach that would increase crop yields in areas where conventional fertilizers are not readily available. The bacterium uses the hydrogen and CO_2 to form a biological plastic that serves as a fuel supply. Once the microbe contains enough plastic, it no longer needs sunshine, so it can be buried in the soil. After drawing nitrogen from the air, it exploits the energy and hydrogen in the plastic to make the fertilizer. Radishes grown in soil containing the microbes ended up weighing 150 percent more than control radishes.

Nocera admits that he initially ran the fertilizer test just to see if the idea would work. He envisions a time, however, when bacteria will "breathe in hydrogen" produced by water splitting and ultimately use the hydrogen to produce products ranging from fuels to fertilizers, plastics and drugs, depending on the specific metabolic alterations designed for the bugs.

A significant breakthrough now solves one of the main limitations of current technologies: a group at the University of Illinois at Chicago managed to directly convert CO_2 into carbon monoxide without the need to use pressurized CO_2; rather it pulls it directly from the air. These scientists employed a transparent capsule, made up of a semipermeable resin membrane and filled with water, to capture CO_2 and then transform it into CO thanks to a light absorber coated with catalysts. They then used the CO to produce a wide range of synthetic fuels, including methanol and gasoline.

This and other innovations in the field of artificial photosynthesis have caught the attention of a new generation of entrepreneurs who are taking these discoveries from the lab to the market, driven by the opportunity to produce fuels from renewable sources. Such is the case for Solistra, a company using patented photocatalysts to produce liquid fuels from CO_2 that deliver more energy than they take to make. Synhelion is using solar concentrators to drive the thermochemical conversion of CO_2 into syngas as a precursor to hydrocarbon fuels. A different approach developed by a young company called Dimensional Energy manufactures environmentally responsible polymers and chemical intermediaries using CO_2 as raw material.

These are just a few examples of how the solar-activated conversion of CO_2 into useful chemicals is already creating business opportunities and companies that have the potential to completely transform how we produce all kind of goods, replacing energy-intensive processes by light-activated chemical reactions that mimic how nature does it.

Javier Garcia Martinez is a professor of inorganic chemistry and director of the Molecular Nanotechnology Lab at the University of Alicante in Spain. He is a co-founder of Rive Technology.

© 2019 Scientific American

Illustration by Cherie Sinnen

© 2019 Scientific American

TAKEOFF: Investigators launch a drone modified to collect airborne microorganisms from a field near Blacksburg, Va.

© 2019 Scientific American

gh-flying microbes

Aerial drones and chaos theory help researchers explore the many ways that microorganisms spread havoc around the world

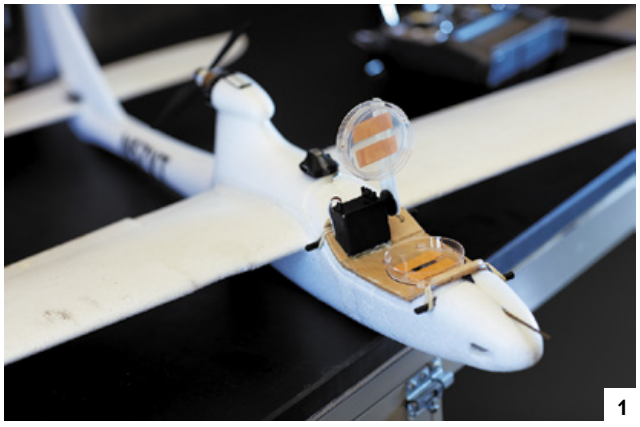
By David Schmale and Shane Ross

Photographs by Adam Ewing

© 2019 Scientific American

The air around us is teeming with microscopic life. With every breath we take, we inhale thousands of bacteria, viruses and fungi.

Scientists have known for almost 150 years that some of these airborne microbes cause disease in plants, domestic animals and people. More recently, we have learned that microorganisms may also affect the weather by allowing water to freeze at warmer temperatures and triggering the onset of precipitation. Astonishingly, a few of these microbes drift on large currents of air to cross oceans and continents. New tools and technology are helping investigators learn more about where these organisms originate, how they spread and the often unexpected ways in which they affect our world during their travels.



1

For more than a decade the two of us have been chasing some of the pathogens that are particularly harmful to agricultural crops, causing billions of dollars in losses around the globe every year from a wide range of ailments, including blight and poisoning by toxins. One of us (Schmale) studies the aerobiology of microorganisms that cause plant disease; the other (Ross) develops mathematical models that describe and predict how currents of air move across short and long distances. We teamed up in 2006 to trace the routes by which plant pathogens spread from one field, region or continent to the next.

To that end (and unique to our collaboration), we deploy a small fleet of airborne drones equipped with sampling devices to collect and analyze the microbes from the lower atmosphere. Every sampling mission turns up a wide range of interesting organisms—many either not well studied or previously unknown to science. We have developed new tools for understanding the long-distance transport of microorganisms in the atmosphere and formed new hypotheses about how far some microbes might travel with the wind and how they might help trigger rain, snow and other forms of precipitation.

Eventually our work may enable agricultural officials to monitor disease-causing microorganisms in the air, predict where they might travel and thus identify which fields to treat or quarantine. Such information will allow farmers to decide, among other things, which crop varieties to plant or when to spray fungicides or other compounds to protect their yields. We have focused much of our research on one pathogen in particular, *Fusarium graminearum,* a fungus that has spread farther and faster over the past few decades than ever before thanks in part to climate change and no-till practices that have increased crop residue in fields, allowing the infection to persist from one year to the next. Whenever agricultural experts, ourselves included, worry that further global warming could significantly threaten the world's food supply in the near future, we are thinking about the explosive spread of this and other fungi that render grains unfit for consumption.

TOXINS IN YOUR FOOD

MANY PEOPLE ARE UNAWARE of just how devastating disease-causing microbes can be to agriculture. One of the worst plant ailments is Fusarium head blight (FHB, commonly referred to as

IN BRIEF

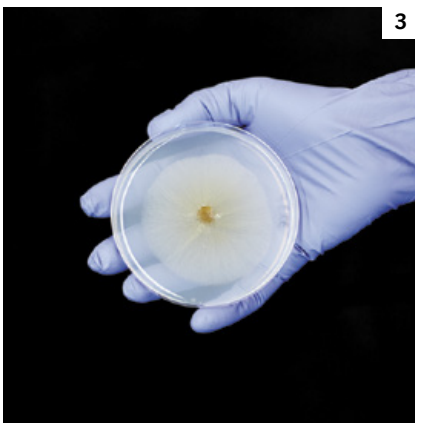
One of the most widely devastating crop ailments is Fusarium head blight (FHB), which primarily affects barley, oats and other small grains and which has been spreading into new regions of the globe in a changing climate.

Because the fungus that causes FHB travels through the air, the authors deployed drones and developed sophisticated simulations to try to determine how far these pathogens can travel. The latest findings show that these microorganisms can be transported by various weather systems for tens to hundreds of kilometers along intricate and ever changing highways in the sky.

The work may eventually help farmers protect their crops by monitoring the spread of plant pathogens and determining the most effective countermeasures.

© 2019 Scientific American

2



3

FLIGHT PLAN: Drones used to study microbes in the lower atmosphere carry specially adapted petri dishes that can be opened and closed from the ground (*1*). A drone flies a preprogrammed route (*2*). And a spore collected from the air grows into a pure culture of *Fusarium* in the laboratory (*3*).

scab), which bleaches the heads of wheat, barley, oats and other small grains and fills the kernels with chemicals called mycotoxins. When ingested in large enough amounts, these mycotoxins make people and livestock very sick, often causing them to vomit. Because grain containing the toxins often cannot be separated from healthy grains, harvested crops must be tested and destroyed if they contain more than a trace amount of toxins.

Several different species of the fungal genus *Fusarium* cause FHB around the world. *Fusarium asiaticum* has long been a problem in central China, from which it has recently begun spreading northward. *F. graminearum* is predominant in the U.S., where it wreaked havoc in corn in the 1970s, causing many pigs to become sick (this outbreak led to the discovery of the mycotoxin deoxynivalenol, which causes pigs to vomit and refuse to eat their feed). Because controlling FHB is so expensive, it has rendered the planting and harvesting of wheat increasingly unprofitable in many states in the U.S. where wheat is commonly grown.

F. graminearum survives winter by hiding out in plants that are left lying on the ground after the previous year's harvest. In the spring and summer, fungal structures called perithecia develop on these residues and forcibly discharge *Fusarium* spores into the air. These spores, in turn, land on the newly emerging anthers of wheat and silks of corn. The spores germinate, and the fungus spreads through the plant, ultimately leading to the accumulation of mycotoxins in the grain. The cross-contamination from one crop to another is why agriculture extension agents advise farmers to avoid planting wheat in fields immediately after they have been used to grow corn or other crops that are susceptible to FHB.

WALLS OF AIR

ONE OF THE GOALS of our collaborative research is to understand how microorganisms are transported over long distances in the atmosphere. As a first step, we decided to measure how far *F. graminearum* can move through the air from an infected field over the course of a day or night.

With funding from the U.S. Wheat and Barley Scab Initiative and the Virginia Small Grains Board, we conducted a series of experiments in commercial wheat fields in Virginia. We took one particular strain of *F. graminearum* that we had isolated from elsewhere in the state and characterized it down to the level of its DNA. In this way, we could distinguish it from the strains that already existed in the fields that we were about to study. Then we spread cornstalks infested with our test fungus over an area about the size of half a hectare and set out a series of petri plates to capture any potential *Fusarium* spores at various distances on the ground from the site of inoculation.

In one set of experiments, we recovered spores from our test strain almost one kilometer from where it had originally been released. But there was no telling how much farther some of the spores might have traveled because one kilometer was the limit of our recovery effort. At any rate, it now seemed clear that *Fusarium* spores could travel much farther than most researchers had previously anticipated.

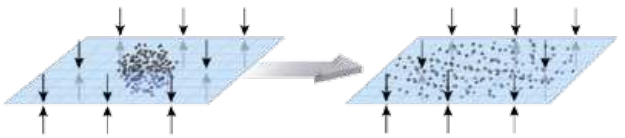
Rather than just continuing to distribute petri plates on the ground farther and farther afield around the state to look for our unique *Fusarium* spores, we decided to search for microorganisms in the air above the fields we studied. The higher up we found the microbes, the more likely we could turn to some of the complex mathematical calculations that meteorologists use to track weather to determine how far they could theoretically travel.

© 2019 Scientific American

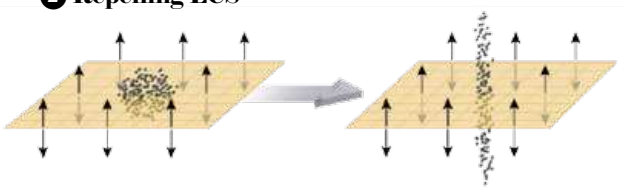
Walls of Air

The movement of air, like that of other fluids, creates certain patterns—such as the Atlantic jet stream—whose shape is influenced by temporary "walls," known as Lagrangian coherent structures (LCSs), made of air. These features fall into two main categories: walls that mostly attract air currents (and any particles they contain) and walls that mostly repel nearby parcels of air. The complex mathematics that governs these structures (*depicted in blue and orange*) determines whether a mass of particles—such as fungal spores—eventually scatters all along the wall's surface ❶ or forms a column on either side of the LCS ❷.

❶ Attracting LCS

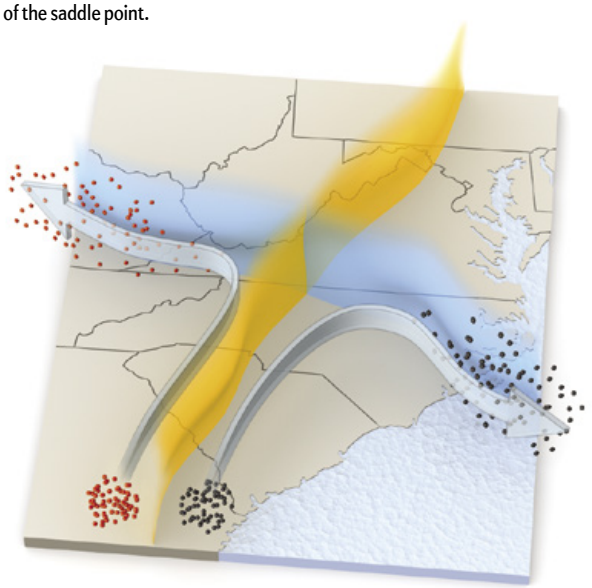


❷ Repelling LCS



LCS Walls Create Different Patterns of Airflow

The intersection of an attracting LCS (*blue*) with a repelling one (*orange*) creates a particular flow of air known as a saddle point. In the example shown, two groups of particles that start fairly close to each other travel hundreds of kilometers in opposite directions once they hit the center of the saddle point.



Thus, we customized a number of drones (unmanned aerial vehicles) with unique sampling devices to collect and analyze microorganisms during flight. With funding from the Emerging Frontiers and Dynamics, Control and System Diagnostics programs of the National Science Foundation, we used the drones to collect some of the *F. graminearum* spores that were already floating over our heads in Virginia. Analyzing the resulting data suggested that some of these fungi had been airborne for several hours—long enough to have been stirred by large-scale weather patterns spanning hundreds of kilometers.

Further investigation revealed that short-lived, invisible moving "walls of air" play a major role in determining how far these fungi travel and where they land. These atmospheric features, formally known as Lagrangian coherent structures (LCSs), arise whenever different currents of air (or any other fluid, for that matter) run into one another or travel around an obstacle, such as a mountain or the wing of an airplane. The currents' initial direction and speed at the moment of contact determine where various air particles will travel next, creating patterns that can be simulated by computers, using the complex mathematics of chaos theory and a specialized branch of physics known as nonlinear dynamics.

As you might expect, these temporary walls of air drive much of the weather we see on any given day. Intricate, ever changing LCS patterns have been shown to shape, concentrate and divide the air over the Atlantic Ocean, for example, in such a way that the winds of a hurricane either gather strength or dissipate as the storm moves over the water. Less extensive interfaces determine how airborne pathogens climb, dive and swirl through a valley, sometimes landing on one farm but not the adjoining property. By tracking LCSs over time and space, we have formulated hypotheses about where various microbial threats to a particular region are likely to come from and where they might go next. As we get better at developing this information, farmers may find it as useful to consult our microbial forecasts as they do the weather report.

Fusarium fungi are just the tip of the iceberg. Because microbes travel through the atmosphere, they clearly do not respect international boundaries. A deadly strain of wheat stem rust (Ug99) has been bouncing around the African continent from its origins in Uganda since the late 1990s; growers in Australia and North America are particularly worried about its potential arrival via regular atmospheric currents over the Indian and Atlantic oceans, respectively. Soybean rust initially rode into the U.S. from South America on Hurricane Ivan in 2004; it currently hides out in the southern U.S. during the winter and makes its annual entry into the Northeast and the Midwest via predictable air routes each growing season (the fungus is unable to survive harsh winters). A coalition of agricultural stakeholders has even established a national monitoring network to keep tabs on this pathogen's seasonal spread every year.

Microbes are not the only particles that ride the wind. Water vapor, air pollution, smoke from wildfires, even chemical agents from a hazardous release, all are influenced by LCSs. Fortunately, our ability to detect and predict LCSs is also improving. During a recent field campaign involving more than 100 participants in the San Luis Valley of Colorado, we used multiple drones flying simultaneously, in concert with ground-based measurements, to do the first experimental detection of LCSs based on wind measurements alone. When a chemical agent is

Illustration by Emily Cooper

© 2019 Scientific American

in the air, LCSs largely determine how it moves and disperses. Our ultimate goal is to use environmental data from swarms of sensors to drive LCS-based transport models of hazardous agents that can lead to proper real-time decisions regarding rapid emergency responses. Using real-time data from unmanned assets, advanced mathematical techniques for speeding up and improving transport analysis and predictive reduced-order models can help assist in emergency response decisions in the future.

HARMLESS FLUORESCENT DYE is released into water. The authors use a drone to image the plume to estimate dye concentrations and how it flows into the environment.

WIND AND WATER

MICROBES DO NOT JUST SPREAD disease while traveling in the sky. They may also help create the weather over land, lakes and oceans. Meteorologists have long known that hail, snow and rain typically fall from the sky only after the formation of tiny ice crystals in clouds. Whether a snowflake or a raindrop forms around the ice depends on certain environmental conditions, including the presence of particles—such as soot—that allow water to freeze at warmer temperatures than usual. (Pure water freezes at temperatures as low as about –38 degrees Celsius.)

Certain strains of *Pseudomonas syringae* produce a particular protein on the cell's surface that traps water molecules in such a way that they start creating a crystal lattice. On the ground, strains producing these ice-forming proteins can cause frost damage to crops. But the microbes can also soar aloft into clouds where the temperature is far below zero degrees C. If enough of these bacteria produced sufficient ice-nucleating proteins in the sky, they could conceivably trigger the formation of raindrops or snowflakes. Whether the microbes are primarily responsible for the onset of precipitation or mostly tagging along for the ride is tough to prove. Ski resort operators are not waiting for a definitive answer, however: many of them use commercial ice nucleators that contain bits of *P. syringae* to create artificial snow during warm winter days.

Supported by the Dimensions of Biodiversity program of the National Science Foundation, research conducted by Schmale and his colleagues has shown that microbes associated with precipitation are far more diverse than originally expected. In Virginia, Boris Vinatzer and Schmale have collected many different types of bacteria and fungi in the atmosphere and in precipitation that can serve as ice nucleators, at least in the lab. And the diversity of microbes associated with precipitation appears to differ depending on geographical location. A better understanding of why each of these microbes predominates in different regions could help us better predict weather patterns. And perhaps we could eventually use some of these microorganisms to develop tools for making it rain in arid regions or areas beset by drought.

Ultimately we hope to combine what we have learned about microorganisms in water droplets with our calculations about Lagrangian coherent structures to describe what happens in the air immediately above the surface of lakes, rivers and oceans. We have collected microbes over water using teams of unmanned boats and aerial drones and have tracked the movement of fluorescent dyes and floating objects in the water to better understand the transport and mixing of hazardous agents such as harmful algal blooms. The mathematical equations needed to describe the mixing of microbe-laden air and water from crashing waves, sweeping winds or even the splashing impact of rain are more complex than anything we have attempted so far. Recent work by Schmale and Sunny Jung of Cornell University showed that spores of a fungal plant pathogen can be liberated by microscopic tornadoes following raindrop impact. Because water covers about 70 percent of the planet, however, we have no doubt that what we find will reveal fascinating new ways that microbes travel the globe. SA

David Schmale is a professor in the school of plant and environmental sciences at Virginia Tech.

Shane Ross is a professor of dynamical systems and fluid dynamics in the college of engineering at Virginia Tech.

MORE TO EXPLORE

Mycotoxins in Crops: A Threat to Human and Domestic Animal Health. David G. Schmale III and Gary P. Munkvold in *Plant Health Instructor.* Published online 2009. www.apsnet.org/edcenter/disimpactmngmnt/topc/Mycotoxins/Pages/default.aspx

Life in the Clouds. Lesley Evans Ogden in *BioScience,* Vol. 64, No. 10, pages 861–867; October 2014.

Highways in the Sky: Scales of Atmospheric Transport of Plant Pathogens. David G. Schmale III and Shane D. Ross in *Annual Review of Phytopathology,* Vol. 53, pages 591–611; August 2015.

Coordinated Unmanned Aircraft System (UAS) and Ground-Based Weather Measurements to Predict Lagrangian Coherent Structures (LCSs). Peter J. Nolan et al. in *Sensors,* Vol. 18, No. 12, pages 4448–4468; December 2018.

Vortex-Induced Dispersal of a Plant Pathogen by Raindrop Impact. Seungho Kim et al. in *Proceedings of the National Academy of Sciences USA,* Vol. 116, pages 4917–4922; March 2019.

scientificamerican.com/magazine/sa

SHANE ROSS

© 2019 Scientific American

Water Made by the Sun

Technologies that pull moisture from the air are now solar-powered

By Donna J. Nelson and Jeffrey Carbeck

BILLIONS OF PEOPLE LACK ACCESS TO CLEAN WATER for all or part of the year or must travel far to collect it. Extracting water directly from the air would be an immeasurable boon for them. But existing technologies generally require a high-moisture climate and a lot of electricity, which is costly and often unavailable. This problem is now becoming more tractable, thanks to robust systems in development that rely on readily available energy from the sun. They are scalable and work even in arid regions—where a third of the world's population lives, often in poverty.

Collaborators at the Massachusetts Institute of Technology and the University of California, Berkeley, have tested an approach that requires no electricity at all. The team intends for its technology to overcome a notable problem with most materials capable of absorbing water from the atmosphere (such as the zeolites in humidifiers): aside from needing high humidity, they give up the trapped water only when heated substantially, which takes energy.

The researchers designed their system around a class of porous crystals called metal-organic frameworks (MOFs), developed years ago by chemist Omar M. Yaghi, now in the U.C. Berkeley group. By choosing specific combinations of metals and organics, scientists can select the chemical properties of each MOF and thereby customize its uses. Beyond their versatility, MOFs' great promise lies with their phenomenally large pores: the surface area inside is almost 10 times that of porous zeolites. For context, one gram of an MOF crystal the size of a sugar cube has an internal surface area approximately equal to the area of a football field.

In April 2017 Yaghi's group, along with that of M.I.T. mechanical engineer Evelyn Wang, reported on a prototype device incorporating MOF-801, or zirconium fumarate, which has a high affinity for water. It pulls moisture from the air into its large pores and readily feeds the water into a collector in response to low-grade heat from natural sunlight. The device can harvest 2.8 liters of water daily for every kilogram of MOF even at relative humidity levels as low as 20 percent, similar to those of deserts. (According to Yaghi, a person needs at least a soda can's worth, or 355 milliliters, of drinking water a day.) Plus, it requires no additional input of energy.

The investigators see more room for improvement. Further experimentation with MOF composition should make the technology less expensive (zirconium currently costs $150 per kilogram), increase the amount of water collected per unit of material and allow researchers to tailor MOFs to different microclimates.

Taking a different tack, a start-up called Zero Mass Water in Scottsdale, Ariz., has begun selling a solar-based system that does not have to be hooked up to an electric grid or an existing water system. Called a SOURCE Hydropanel, the system uses both solar photovoltaic and solar thermal to drive air through a proprietary water-absorbing material and condenses the extracted moisture into fluid. A small lithium-ion battery operates the device when the sun is not shining. Each Hydropanel, the company says, can produce an average of 60 to 150 liters of liquid water a month, which is stored in a 30-liter reservoir that adds calcium and magnesium for health and taste.

Cody Friesen, founder of Zero Mass Water and a materials scientist at Arizona State University, developed Hydropanels with the aim of having them work sustainably and easily anywhere in the world. An installed residential array sells in the U.S. for about $6,500. The company partners with nonprofits to build arrays of the technology in parts of the globe lacking water infrastructure. The same Hydropanels reducing the need for bottled water in the U.S., Friesen notes, can also provide clean water to a school that lacks it so kids "are able to get educated and not get sick."

Over the past few years, he says, Hydropanels have been installed across the U.S. and 22 other countries—among them Mexico, Zimbabwe and Australia—and the company has recently completed a project funded by the U.S. Agency for International Development to provide water to Syrian refugees in Jordan and Lebanon. When most people think about solar, Friesen adds, "they think about electricity. In the future, they will think about water abundance." SA

Donna J. Nelson is a professor of chemistry at the University of Oklahoma and in 2016 was president of the American Chemical Society. She was a scientific adviser for the television show *Breaking Bad*.

Jeffrey Carbeck has spent more than 20 years in the fields of science and engineering. A former professor of chemical engineering and molecular biology at Princeton University, he is currently CEO of 10EQS, a global consulting firm that leverages the power of business insights. He has a Ph.D. in materials science from the Massachusetts Institute of Technology and did a chemistry postdoc at Harvard University.

© 2019 Scientific American

Illustration by Eric Petersen

© 2019 Scientific American

© 2019 Scientific American

Earthquakes in the Sky

The best early warnings of a big disaster may appear 180 miles above the ground, a controversial new theory says

By Erik Vance

Illustration by María Corte

© 2019 Scientific American

ON FRIDAY AFTERNOON, MARCH 11, 2011, KOSUKE HEKI was in his office in Hokkaido University in northern Japan when the ground began to shake. The pulses were far apart, and each one lasted a few seconds. Heki, a geophysicist who studies an arcane phenomenon involving odd patterns formed by electrons in the sky after quakes, was interested but not unduly alarmed. It seemed like a large earthquake but far away. As the shaking continued, he thought perhaps data from the event might help his research. Then someone flipped on the news, and Heki's curiosity turned to horror.

The waves he felt had come from the biggest temblor in modern Japanese history—the devastating magnitude 9.0 Tōhoku earthquake, which cost the country hundreds of billions of dollars and claimed more than 15,000 of his compatriots' lives. The tsunami after the quake crippled the Fukushima Daiichi Nuclear Power Plant and triggered the worst nuclear disaster in a quarter of a century.

While emergency personnel worked to evacuate people and save lives in another part of the country, Heki could only wait for spotty phone and Internet service to come back online. By Sunday, the Internet was working, and he quickly downloaded satellite observations of the air over the region of Tōhoku and hungrily combed through them. As he expected, electrons in the ionosphere showed a disturbance 10 minutes after the quake. But he could not get his model to fit the data by just looking at the minutes after the quake. So he tried expanding the time frame, including the hour before. That is when he saw something that stopped him in his tracks.

Forty minutes *before* the earthquake struck, there was a subtle rise in electron density above the temblor's epicenter. Maybe it was an anomaly, a one-off or an instrument malfunction. Or maybe it was something more. Scientists have yet to find a reliable earthquake precursor—a telltale sign that could alert people before the onset of a large quake. If electron changes were such a warning, they could save thousands of lives a year.

Heki, whom colleagues describe as unassuming, quiet and cautious, was immediately skeptical of his own data, so he pulled up information from two other earthquakes. He saw the density change again and decided to keep digging. To date, he has found the electron signal before 18 big quakes, and over the past eight years he has come to believe it is real.

Other experts are now starting to take a close look at the idea. "Years ago people didn't think we could predict the weather, but we do now," says Yuhe Song, an expert in remote sensing at NASA's Jet Propulsion Laboratory. "We probably can see something earlier than when we feel it on the ground. There is something there ... I think this warrants a discussion."

Not everyone agrees. Many scientists see Heki's work as the latest in a long line of false prediction promises. "These things are like the common cold: they're always going around," says seismologist Robert J. Geller, an emeritus professor at the University of Tokyo, who has spent years debunking various earthquake forecasting ideas. "If you ignore them, they go away."

Heki's idea seems to be sticking around, however, and may be getting stronger. The electron signal has shown up in medium-sized quakes as well as the largest ones. Other scientists have formed a theory that connects faults in the ground to activity in the sky. Heki has published his findings in reputable journals such as *Geophysical Research Letters* and been invited to lecture about the results at the American Geophysical Union's annual meeting. In 2018 Japan's Chiba University hosted an entire meeting to debate quake prediction, including his idea. If Heki is right, the implications for public safety are enormous, but there are difficult questions about how to use such a precursor. How accurate must a warning system be to sound an alarm, and what kind of emergency response should ensue?

IN BRIEF

Tens of thousands of people can be killed by a single earthquake, so scientists have struggled to predict quakes well enough to sound an alarm.

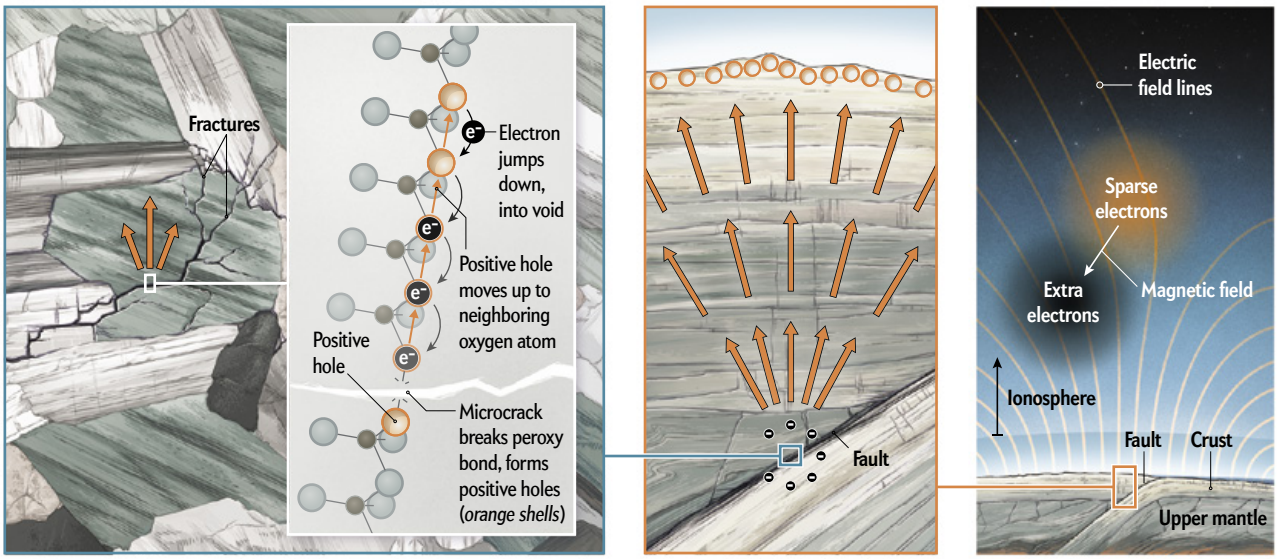
New observations suggest that clumps of electrons form in the ionosphere, sometimes 30 minutes or more before a temblor, giving an early warning.

There have been false promises of prediction in the past, so this notion is drawing skeptics—but the data are beginning to convince more scientists.

© 2019 Scientific American

From the Ground Up

Electrical disturbances miles above the planet's surface may occur at least half an hour before major earthquakes, new research indicates. These could be early warnings of disasters. And there is a theory about the way cracks in rocks might create activity high in the sky.



1. A Fracture Begins

Within the ground, parts of the earth's crust slide slowly across one another. Sometimes at a fault line they jerk suddenly, and the strain of the movement begins to tear the rock apart, creating small breaks called microfractures.

2. Electrons Jump

The microfractures generate enough force to break peroxy bonds, which hold together oxygen atoms within molecules in rock grains. This force alters the energy of negatively charged electrons in these grains, making the electrons move. They leave behind positively charged spaces called holes. As more electrons move, the holes move in the opposite direction, creating a tiny electric current in the rock grain.

3. To the Surface

This process continues across adjoining grains of rocks, like chains of falling dominos. Electrons move, leaving room for holes and their positive charges to propagate up from the original fracture, jumping from grain to grain up to the surface. Behind them, the strain created by grinding rocks grows.

4. Up in the Air

When positive holes accumulate at the surface, they can pull electrons from molecules there, generating an electromagnetic field. These fields can form lines that extend miles upward. They alter patterns of electrons in the ionosphere, making dense clumps in certain spots and sparse concentrations in others. Such anomalies can be detected by satellites.

PREDICTING THE WORST

CHARLES F. RICHTER—creator of the quake magnitude scale that carries his name—is said to have remarked that "only fools and charlatans predict earthquakes." But that hasn't stopped people from trying. In 373 B.C., animals reportedly ran for shelter five days before an estimated 6.0 to 6.7 magnitude temblor rocked Greece and destroyed the city of Helike. The Japanese once thought that twitching or thrashing catfish could predict earthquakes. Dogs, sheep, centipedes and a Sumatran pheasant called the great argus have all been said to change their behavior before a quake.

Others have looked at wells that suddenly go dry, temperature changes, radon gas emissions and, of course, groups of smaller foreshocks as possible precursors. In 1975, using a combination of these signs (including animal behavior), the Chinese even managed to predict a 7.3 quake early enough to begin evacuating the city of Haicheng. It raised hopes. "In the 1970s American and Japanese seismologists became pretty optimistic about short-term earthquake prediction," says Masao Nakatani, an expert in rock mechanics at the University of Tokyo. "We tended to believe that earthquakes must be predictable." By the 1980s both the U.S. and Japan had created research groups to pursue the challenge.

Reliable signals proved elusive, however. One year after the Chinese success the same techniques failed to spot another, larger quake that killed hundreds of thousands of people. Japan, sitting on the tectonically restless Ring of Fire around the Pacific, put in a fair amount of effort only to find that a precursor would work once and not again. Nature seemed to keep changing the rules. The U.S. abandoned forecasting efforts in the late 1990s after a predicted quake—based on the pattern of previous earthquakes—failed to appear near Parkfield, Calif. (It eventually hit in 2004 but with none of the expected warning signs.)

The year of the Tōhoku quake, an international commission on prediction, set up by the Italian government, essentially closed the book on the field. "In spite of continuous research efforts in Japan, little evidence has been found for precursors that are diagnostic of impending large earthquakes," the members wrote in May 2011.

Illustration by Matthew Twombly

© 2019 Scientific American

Four months later Heki reopened the book. What he saw were bizarre pockets of ionized particles not at or on the earth's surface but 186 miles above it. The idea of a connection between ground and sky is not out of this world. In the 1970s scientists first found that rocks under extra pressure create an electric current, like a very weak battery. The theory goes that as a rock is pressurized, its oxygen atoms give up electrons, leaving deficits that physicists describe as positive holes. Electrons from other nearby atoms move into those holes, leaving yet more holes behind them, creating a chain reaction of moving charges.

The holes "have the ability to move around over long distances—miles, tens of miles, hundreds of miles," says Friedemann Freund, a researcher at NASA and the SETI Institute, who discovered the phenomenon. "It's like a bucket of water in a fire line. It's being handed from person to person to person."

TOLL OF A QUAKE: With little warning, the deadly Tōhoku earthquake and tsunami destroyed the Japanese city of Rikuzentakata; afterward, residents walked among the ruins.

NICOLAS ASFOURI *Getty Images*

Freund says that the holes then roam through rocks, eventually reaching the earth's surface, where they attract negatively charged electrons from molecules in the air, like a magnet attracting iron shavings. The electrical charges then travel to the upper atmosphere. The mechanism is just theory because it is hard to measure directly, but it seems to fit with hints of electron clumps seen after an earthquake. But no one had clearly seen the effect *before* a quake.

For his research, Heki brought in a new method that used sophisticated GPS satellite networks, which can detect subtle changes in atmospheric electrons when their radio signals bend across the atmosphere. Japan has a particularly dense GPS receiver network, which allowed Heki to spot a subtle electron surge in the sky far above Tōhoku's epicenter, about 40 minutes before seismometers in the ground detected any movement.

But the geophysicist says he was reluctant to present his findings. "I had to worry about how to publish it," he says. "Earthquake prediction is something special. Everybody becomes very emotional."

He did not, in fact, publish right away. After Tōhoku, Heki looked back at two giant earthquakes where detailed GPS data were available. In each, he found a telltale rise in electron concentration more than 30 minutes beforehand. The larger the quake, the longer the advance time, it seemed. A magnitude 8.2 quake in 2014 in Chile had a 25-minute lead time, whereas 9.0 Tōhoku gave the 40 minutes. So the signals not only hinted that the faults were about to slip; they also indicated the relative size of the ensuing temblor. "I have never seen such a clear phenomenon occurring just before an earthquake," he says.

CHAOTIC DEBATE

ARMED WITH THESE DATA, Heki finally published a paper in September 2011, announcing what he found. Other scientists quickly started pointing out problems. Some said the result came from a misreading of the data and that disturbances during and after the quake muddied the picture. Heki responded by using a different analytical method to highlight the prequake effects. He also converted measurements taken at an angle to a bird's-eye view, thinking this would make the effects easier to spot. But critics argued this was just reorganizing the same flawed data. Another Japanese team said the effect was caused by geomagnetic storms. Heki performed another analysis to account for storm effects and found that storms could not explain all the changes he saw.

Soon some doubters began to agree with him. "This is by far the best precursor ever reported," says Nakatani, who says he stopped believing in earthquake forecasting after the failures of the 1990s. But Heki has rekindled his faith, so much so that he now says the work could very well be "the most important discovery in the history of earthquake science." NASA's Song is less hyperbolic but agrees the electron clouds have been hard to explain away as errors and seem to signify a real event. Freund says Tōhoku followed months of pressure buildup and changes in electron density. And although that pressure might have found other outlets—such as invisible "silent" earthquakes—the charged particle release is still a predictable phenomenon that, in theory, could be detected in other quakes.

Critics, however, insist Heki is seeing things in a computer that do not exist in the real world. "He is trying to confirm his initial thought without providing a valid support," says Fabrizio Masci of the National Institute of Geophysics and Volcanology in Italy. He has published papers refuting not just Heki but other earthquake prediction ideas and says Heki's responses are "a skillful way to distract the reader." Many of the criticisms focus on Heki's reading of baseline electron levels. The tiny particles permeate our planet and fluctuate as much as the weather. Heki says that just before an earthquake, electrons clump a little

© 2019 Scientific American

more than average. Critics say that the change is caused by the daily ebb and flow of electrons. In other words, Heki may be chasing a statistical ghost.

Masci goes even further and says seismic precursors might be impossible if earthquakes themselves are fundamentally chaotic. If the initial conditions of an event are not precisely determined, it is impossible to know how the effects will play out. And with quakes, it is devilishly hard to nail down all the initial conditions.

Giovanni Occhipinti of the Institute of Earth Physics of Paris is not so pessimistic, although he agrees it is a daunting problem to understand all the factors at play—the rock type, the pressure, the faults nearby—well enough that you can make a prediction. Occhipinti, like Heki, studies how earthquakes affect atmospheric ions. He says that, given how chaotic ions are in the atmosphere, you simply cannot pull a signal from all the noise. It is like trying to predict a hurricane based on a single cloud a day beforehand. "The problem is there are tons of clouds that are coming and moving around," he says. "It's not simple to deduce a way to discriminate that specific cloud that you want to see as a precursor."

"IT'S PUSHING SCIENCE FORWARD," BUT "YOU HAVE TO BE REALLY, REALLY, REALLY PRECISE. YOU ARE PLAYING WITH THE LIVES OF PEOPLE."

—Giovanni Occhipinti *Institute of Earth Physics of Paris*

Until recently, Occhipinti was on the side of skeptics and felt that Heki's discovery was merely a statistical hiccup. Heki's latest work, however, which takes into account the complex 3-D space in which the effects happen, caught his interest. Rather than a limited satellite snapshot, 3-D modeling shows multidimensional effects that point to a consistent physical process underlying the anomalies, making them hard to write off as ghosts. Occhipinti wants to see more 3-D analyses, along with comparisons of those results with other models to see how well they fit. So he is not, as yet, a complete believer. But he calls the idea "intriguing" and is now looking into it more closely. "It's pushing science forward," Occhipinti says, but "you have to be really, really, really precise. You are playing with the lives of people."

SOUNDING ALARMS

THE NUMBERS OF THOSE LIVES can reach into the hundreds of thousands. The U.S. Geological Survey examined worldwide earthquake fatalities for a 16-year period beginning in 2000. The death counts fluctuate because there are not giant quakes every year. But the toll is daunting. In seven of those years there were more than 20,000 deaths, and for another two years the totals exceeded 200,000. In the countries hardest hit, people are desperate for any kind of warning, even just a few seconds. Take Mexico City, one of the most lethal and well-studied earthquake zones on the planet. After a devastating 1985 quake that killed as many as 10,000 people, the government took advantage of the fact that quake waves travel over unusually long distances in the region and built a monitoring system that can give a couple of minutes warning if the waves are detected far enough away.

Carlos Valdés, a geophysical engineer and director of Mexico's National Center for Prevention of Disasters, says a 40-minute warning might sound good, but the reality is not so simple. First, false alarms can ruin any emergency response. Some Mexican quakes triggered warnings but were too weak or in the wrong position to actually shake the city, for instance. People became annoyed and stopped responding to those alerts. But he worries more about the opposite problem: panic. "Somebody is going to say, 'I have 40 minutes, I'm going to leave the city,'" he says. "It takes only one person to start screaming or start running, and everyone follows." Roads clog, and no one gets to safety.

Still, other emergency planners note that even short warning times create the opportunity to shut down gas lines or stop subways, reducing risks. And greater accuracy would solve the false alert problem. British and Russian scientists have proposed a satellite that could better track atmospheric anomalies such as the ones Heki studies, and China is moving forward with a space-based prediction program that relies on electromagnetic disturbances in the ionosphere. But given the complex nature of the ionosphere, coupled with the confusing nature of earthquakes, it may be decades until atmospheric data become actual earthquake warnings.

Geller does not think that day will ever come. "The precursor hunters throughout the past 130 years have a childlike belief that, one, there must be precursors and that, two, the bigger the quake, the bigger the precursors must be. But there's no particular reason these beliefs should be correct," he says.

Still, Heki is moving forward. He recently published a paper that analyzes the precursor of a 2015 Chilean quake in detailed 3-D, which he says may make his ideas harder to refute. He is also trying to fill in some data gaps between the electrical charges and the actual earthquake locations themselves. The goal is to better understand what it is in the crust that creates the effects high above. "There is something before an earthquake in the ionosphere. I don't know about a physical mechanism," Heki says, "but the observation itself is so clear." SA

Science writer **Erik Vance** wrote about vaquitas, threatened porpoises in the Sea of Cortez, in the August 2017 issue of *Scientific American*. He lives in Baltimore.

MORE TO EXPLORE

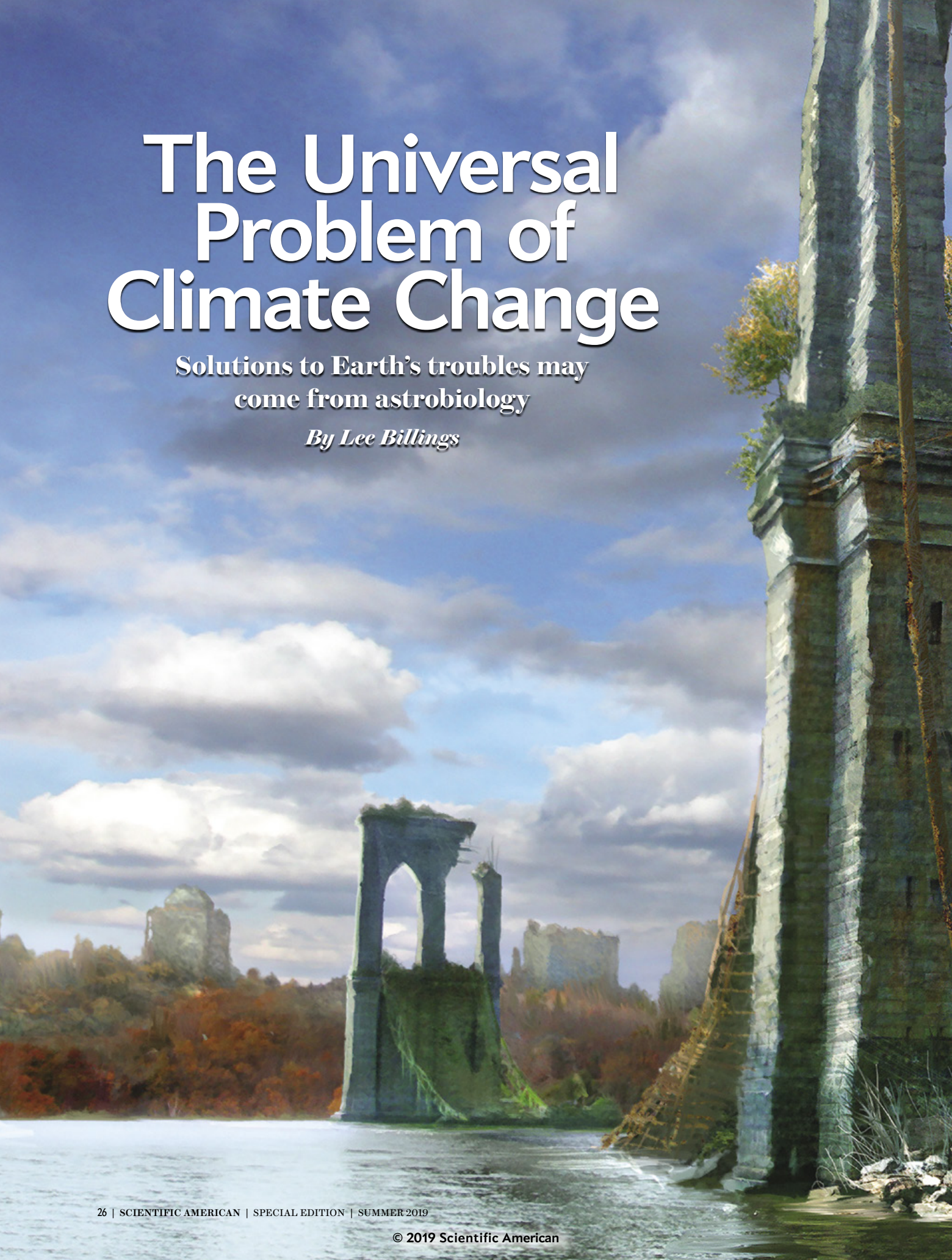
Apparent Ionospheric Total Electron Content Variations Prior to Major Earthquakes Due to Electric Fields Created by Tectonic Stresses. Michael C. Kelley et al. in *Journal of Geophysical Research: Space Physics,* Vol. 22, No. 6; pages 6689–6695; June 2017.

Ionospheric Anomalies Immediately before M_W7.0–8.0 Earthquakes. Liming He and Kosuke Heki in *Journal of Geophysical Research: Space Physics,* Vol. 122, No. 8, pages 8659–8678; August 2017.

Three-Dimensional Tomography of Ionospheric Anomalies Immediately before the 2015 Illapel Earthquake, Central Chile. Liming He and Kosuke Heki in *Journal of Geophysical Research: Space Physics,* Vol. 123, No. 5, pages 4015–4025; May 2018.

scientificamerican.com/magazine/sa

© 2019 Scientific American



The Universal Problem of Climate Change

Solutions to Earth's troubles may come from astrobiology

By Lee Billings

© 2019 Scientific American

DARK FUTURES: Results from modeling the co-evolution of technological civilizations and their planetary climates suggest that aliens could, much like human societies in Earth's recent history, suffer self-inflicted collapses caused by rapid environmental degradation.

© 2019 Scientific American

MUCH HAS BEEN WRITTEN ABOUT THE ANTHROPOCENE—A PROPOSED new division of geologic time in which humans are a dominant force for planetary change: When did it begin? How might it unfold? And can we, the supposed masters of Earth, actually use our powers to make our planet a better place?

Understandably, most of the Anthropocene's literature to date both in the popular press and in peer-reviewed publications has been decidedly Earth-centric. Yet in a recent series of papers and a new book, *Light of the Stars: Alien Worlds and the Fate of the Earth,* astrophysicist Adam Frank argues that the Anthropocene's origins and implications are best understood in the context of astrobiology, the study of life in the universe. Climate change and other environmental effects associated with humankind's global ascendance, he says, most likely are universal phenomena for any and every technological civilization that emerges somewhere in the cosmos. Which means the most crucial insights governing the Anthropocene may come less from studying the ground beneath our feet and more from turning our gaze to the heavens.

SCIENTIFIC AMERICAN spoke with Frank, a professor at the University of Rochester, about the lessons to be learned from speculations about alien civilizations battling climate change. An edited transcript of the interview follows.

What motivated you to write this book?

The book was inspired by some frightening conversations I ended up having with climate change denialists in response to my pieces on that topic for National Public Radio and the *New York Times.* It was terrifying, really, to see how locked into their perspective these people are and to realize there's this false narrative about climate change, which we're stuck in. So this was motivated, in part, by my thinking about how to change the discussion.

Over the years what I've come to understand is that human-driven climate change is really an astrobiology problem. It's not a problem of politics. It's not a problem of businessmen versus environmentalists. We are talking about something much bigger—a planetary transition, which some scientists label as the Anthropocene. Climate change is just one aspect of this new human-dominated period. My argument is that Anthropocenes may be generic from an astrobiological perspective: what we're experiencing now may be the sort of transition that everybody goes through, throughout the universe. And there are probably some common features to long-lived civilizations and the planets they inhabit.

I really started exploring this in 2014, when I co-authored a paper with Woody Sullivan of the University of Washington that proposed using dynamical systems theory to model some of these planetary transitions. We argued that it's possible to identify the basic paths that "exocivilizations" might follow and the feedbacks that might occur when they begin altering their planetary climates. In my latest paper, published with several colleagues, we went ahead and actually did some of that modeling.

Why would you have any faith in models examining the behaviors of exocivilizations—something no one has ever seen?

I like to draw a parallel to the Higgs boson. This is a fundamental particle that was "discovered" in 2012, but really you could say it was discovered in 1964. That was when three papers appeared extrapolating from well-understood physics to propose this particle that would wait nearly a half-century before actually being seen. The details obviously would have to be filled in by actual data, but in that intervening time physicists went quite far in thoroughly extrapolating the particle's nature.

Thus, when it comes to thinking about the interactions between an advanced technological civilization and its planet, well, we actually know a lot more about that today than people knew about the Higgs boson 50 years ago. We have lots of examples of planetary climates that we've studied right here in the solar system—Venus, Mars, Titan, Jupiter, and so on. And we've got computer models that can nicely forecast, for instance, the weather on Mars! So we really do understand climate pretty well. And a civilization, to some degree, is just a mechanism for transforming energy on a planetary surface. This gets us into the realm of thermodynamics on global scales, which is supercool.

As long as we ask the right sort of question—"What is a planet's response to having this energy dumped into it?"—we have reasonably good "guardrails" that allow us to address it.

But that's mostly planetary science. What about the social or biological aspects here? How are you modeling that?

Well, just as we understand planetary climates pretty well, we can use the basic, fundamental tenets of life to guide us, too. Organisms are born, some of them reproduce and they die. Living things consume energy, and they excrete waste. That should be true even if they're made of silicon or whatever.

The next step is to incorporate principles of population biology, in which the idea of "carrying capacity"—the number of organisms that can be sustainably supported by the local environment—is very important. This approach can also be mathematically applied to the state of a planet. So in our latest modeling work we've got an equation for how the planet is changing and an equation for how the population is changing. What ties them together is the predictable result that as environmental conditions on a planet get worse, the total carrying capacity goes down. A civilization with a population of n will use the resources of its planet to increase n, but at the same time, by using those resources it tends to degrade the planet's environment.

In our study, we used these basic ideas to address the question of if, and how, exocivilizations can get through their own versions of the Anthropocene. Our first models were pretty simple but gave us a pretty rich view of the possible trajectories for exocivilizations.

What were the results of your modeling? And do they make you feel optimistic or pessimistic about our own prospects?

In the models we saw three classes of behaviors, three trajectories: A "die-off," where the population overshoots the carrying capacity and then dwindles; a "steady state," where the population growth slows and ends up within the bounds of carrying capacity; and a "collapse," where the population and the carrying capacity both just drop like a stone.

It's important to remember we aren't trying to say any particular history is bound to play out on any particular planet.

PRECEDING PAGES: KENN BROWN AND CHRIS WREN *Mondolithic Studios*

© 2019 Scientific American

We are looking at the average properties of "successful" versus "unsuccessful" trajectories for civilizations reaching this Anthropocene-like planetary transition.

The results actually made me feel both optimistic and pessimistic. I was happy to see we did find sustainable steady states—it could've turned out that they didn't exist. So our models predicted that long-term civilization/planet co-evolution is possible. Hooray!

But we also found trajectories where equilibrium could be reached only after 90 percent of your population died off. It's not even clear that a complex technological civilization could survive losing nine out of every 10 individuals. It might well just descend into chaos.

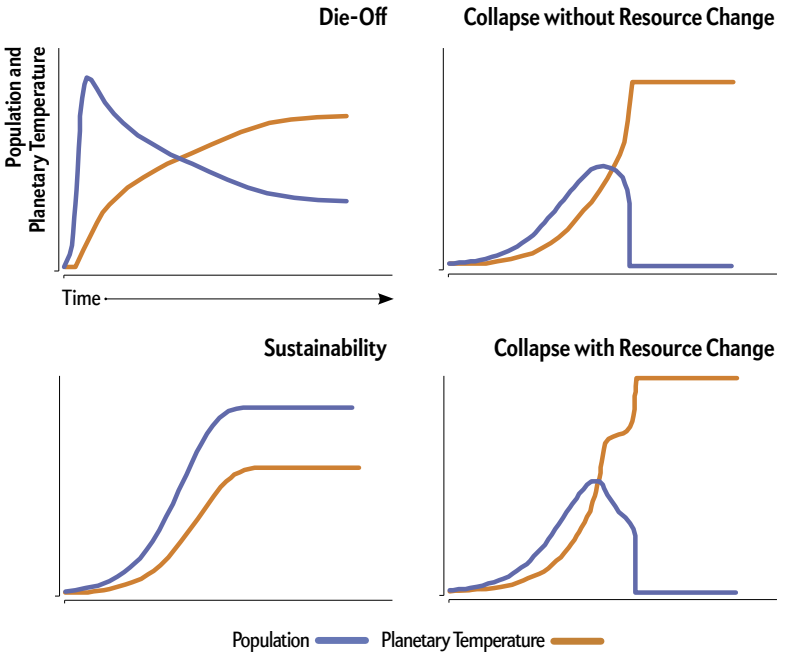
And there was also a fourth and really chilling trajectory we found. It popped up when we looked at how a civilization might switch from high- to low-impact energy sources—such as switching from fossil fuels to solar power, for instance. In some of those scenarios, where the population is soaring and the planet is heating up, the civilization shifts to the low-impact energy resource, and everything seems to get better at first—but then a collapse occurs anyway. This fourth trajectory is really scary because it suggests you can make all the right choices and still have things not work out.

Why would that happen?

Because planets are complex systems that exhibit nonlinear dynamics. Messing around with a planet's climate can be like rolling a big rock down a hill. Once the rock really gets going, there's no turning back, and maybe it just shoots right off a cliff.

The climate is basically a giant planet-sized machine with lots of moving parts—the atmosphere, the lithosphere, the cryosphere, the biosphere—and the interactions among those moving parts may be quite sensitive. So if you push it hard enough, even if you stop pushing it shortly after, there isn't always a way to recover. That's one of the big messages of the book. We have to start thinking like a planet. Our view of climate change—the faux political debate—is so narrow because we think we're the first time this has ever happened. We're kind of like cosmic teenagers who are stuck in our own immaturity.

FOUR SCENARIOS for the fate of technological civilizations and their planets, based on mathematical models developed by Adam Frank and his collaborators. The blue line plots the trajectory of the civilization's population, and the orange line shows the co-evolving trajectory of the planet's temperature (a proxy for climate).



SOURCE: "THE ANTHROPOCENE GENERALIZED: EVOLUTION OF EXO-CIVILIZATIONS AND THEIR PLANETARY FEEDBACK." BY A. FRANK, J. CARROLL-NELLENBACK, M. ALBERTI AND A. KLEIDON, IN *ASTROBIOLOGY*, VOL. 18, NO. 5; MAY 2018

So where do you think Earth is in this spectrum of possibilities?

I don't think anyone knows yet. More work needs to be done—better models with more realistic climates. There has certainly been a lot of discussion in the Earth science literature about planetary "tipping points," the idea that you push too far and, whoops, now you're tipped over to another state. Clearly, people worry about this. But it's unclear as to where and what exactly the tipping points are and where we may be relative to them.

But back to the idea of "changing the discussion"—your motivation for writing the book. Surely we could do more with these ideas than just discuss them. Don't they recommend some action?

Of course, they do—but most of those actions are indistinguishable from ones that follow from lots of other work in climate science and policy.

I really do think, though, that the route to our making it through the Anthropocene runs through other planets. We aren't going to become a sustainable planetary civilization by only dealing with Earth. I give lots of examples in the book, but one of the best is the fact that the climate models that revealed the possibility of "nuclear winter"—a global cooling caused by the atmospheric effects of a nuclear war—relied heavily on data about Martian dust storms. Talk about cross-fertilization! Those nuclear winter models totally changed the debate about nuclear weapons, and they came from understanding another world.

So we can't just sit here studying tipping points on Earth at the expense of going out and exploring other worlds and looking for life and intelligence elsewhere, because the knowledge we gain from that is probably essential for our own future. Think of it this way: If you're sick and go to the doctor to be cured, the doctor will have pretty limited abilities if he has only ever studied [just] you. If he has studied you and lots of other people, [however,] he'll have a much clearer picture of what ails you. It's probably like that with planets and civilizations, too. SA

Lee Billings is a senior editor for *Scientific American*. He covers space and physics.

© 2019 Scientific American

ADAPT OR DIE:
Sitka spruce in British Columbia may need to borrow genes from trees in warmer climates.

© 2019 Scientific American

FORESTS ON THE MARCH

Trees can't walk to a better place as climate worsens. So scientists are relocating helpful genes instead

By Hillary Rosner

© 2019 Scientific American

IN A FIELD IN VANCOUVER, ACROSS THE ROAD FROM A ROW OF TIDY WHITE TOWNHOMES, roughly 500 bushy Sitka spruce trees climbed toward the sun. On a spring day in 2013 the trees, triangle-shaped with tightly packed, deep-green needles, were crammed shoulder to shoulder—or, in some cases, shoulder to waist. Although the spruces were all planted at the same time, seven years earlier, their height varied like primary schoolchildren assembled for a group photograph.

The smallest trees, around two feet tall, hailed from Kodiak Island, Alaska; the tallest, at nearly seven feet, originated in Oregon. Height was not the only visible difference. The spruces from Alaska produced buds a full three months earlier—an entire season—than those from Oregon. The Alaska trees also stayed green and healthy no matter how low the temperature dropped.

The spruces had been rooted in this field, at one end of the University of British Columbia's rambling campus, as an experiment to highlight how trees adapt to local environments. That trees match their habitats may sound obvious. But the details are important because of a looming threat: those habitats are changing as the planet warms—and trees can't exactly get up and walk to a new home. If a species cannot keep pace with a changing climate, it is doomed.

Because the trees themselves cannot relocate, scientists are exploring a novel solution: relocating the plants' DNA. That is why Sally N. Aitken planted the spruce garden. Aitken, director of the university's Center for Forest Conservation Genetics, believes saving the forests of British Columbia—and others around the world—may hinge on a practice called assisted gene flow. It could help species adapt to future conditions by moving organisms with particular traits from one part of their natural range to another. The tree from Oregon and the tree from Alaska just might have some genes that could help each other out. But without intervention, they would never meet.

Like an arboreal matchmaker, a forester could take seeds from spruces or lodgepole pines at a low elevation, say, and plant them farther upslope. As temperatures on the higher slopes warmed, the relocated trees would grow up and breed with their local counterparts, spreading their warm-adapted genes throughout the area and thus helping the forest adjust. Assisted gene flow could give species an evolutionary hand.

But you can't simply take a tree from Oregon, plant it 1,000 miles away in northern British Columbia and wait for the mercury to rise. The reasons that you can't come from the same local genetic adaptations that make the gene-flow idea attractive. Lodgepole pines, for instance, grow in different regions throughout much of the Canadian province. Their genes help some trees better tolerate heat or cold or drought or fend off local diseases or pests. If an Arctic cold front moves through Vancouver, hitting transplants from warmer regions, they will suffer. "We need to shift these things starting with baby steps," Aitken says. "The changes projected over the next few decades are really big, but we still have a lot of year-to-year and week-to-week and month-to-month variation that those trees have to survive."

Figuring out how to match today's seeds with tomorrow's climates is no easy task. But in British Columbia, where forestry accounts for a third of all exports and commercial forests make up nearly half of the total forest cover, it is vital. Provincial law requires that forests be replanted after logging, to bolster future timber supplies and healthy ecosystems. Roughly 250 million seedlings are planted annually. Just where those seeds should come from and how far they can or should be moved is a complicated—and pressing—question. Do it wrong, and you could be dooming the forests for decades to come.

The Sitka spruce experiment, which involved trees from 14 different locations ranging from central California up to Alaska, was Aitken's small proof of concept for a larger effort to avoid this type of fatal misstep. The research yielded 35 segments of DNA associated with cold tolerance and bud timing. Next Aitken and her

IN BRIEF

Forests adapt genetically to survive in local conditions, but climate is changing faster than trees can adjust.

To prevent forest death, scientists are moving trees with genes tied to water use and heat tolerance near other trees that need this DNA so they can breed.

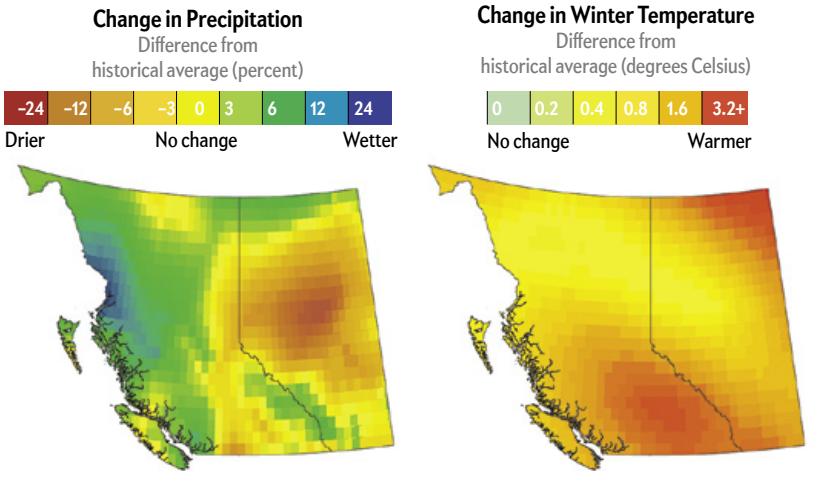
Assisted gene flow, as the strategy is called, is being tested on trees from different climate zones in British Columbia.

PRECEDING PAGES: TJ WATT

© 2019 Scientific American

Weather Trends for Trees

Scientists compared the past with the present to figure out recent climate trends in British Columbia and Alberta and whether they depart from what trees experienced during the latter half of the 20th century. Researchers gathered direct observations from weather stations throughout the provinces between 1961 and 1990. The scientists compared this baseline with averages from a more recent period, 1997 through 2006. For the recent period, they noted whether the averages were wetter, drier or warmer or did not change from the earlier averages. They learned that the climate has become much wetter along the Pacific coast, which the researchers link to outbreaks of previously rare pine needle blight. At the same time, it has become drier in the interior, which may account for spruce and aspen deaths there. Winters have been warmer all over the provinces, allowing the tree-killing mountain pine beetle to spread to more forests. (Methods for this analysis were published in *Agricultural and Forest Meteorology* in 2009.)



team sifted through more tree genomes to find genes responsible for proteins that are linked to other environmental traits. By examining these genes and comparing them among species (lodgepole pine and two varieties of spruce), they were able to show that closely related species adapted in similar ways using the same genes. Their hope is that beneficial versions of these genes—called alleles—would spread through populations that need the traits, in rough synchrony with the climate changes that make those genes useful.

The project, called AdapTree, as well as a more recent effort, CoAdapTree—which looks at three more species of conifer—could help pave the way for future assisted gene-flow projects around the world, work that could in turn help other species that are key ecosystem architects. Started in the fall of 2016, the project looks not just at climate adaptation but also at two tree diseases. "Insects and diseases are increasing their impact as the climate warms," Aitken says. The effort is co-led by a forest pathologist, Richard Hamelin, and is examining both tree responses to the diseases and the ways the pathogens themselves vary genetically.

Aitken and Michael C. Whitlock, a population geneticist in the university's zoology department, coined the phrase "assisted gene flow" in a 2013 paper. Over the past decade scientists and conservationists have been arguing over a more grandiose idea, "assisted migration," which generally refers to moving species greater distances, outside their natural ranges. But assisted gene flow within a species' range is a more measured approach with genetic rigor at its heart. By the time AdapTree wrapped up, scientists had assembled DNA-sequence information for 12,000 lodgepole pine and spruce trees from more than 250 populations across British Columbia and Alberta.

CLIMATE ZONES

THOSE TREES are already feeling the effects of a changing climate. Back in the 1970s, British Columbia's government produced a climatic map of the province, organizing it into a series of biogeoclimate zones. That map has underpinned forest planning in western Canada for four decades, helping to govern which seeds can be planted where. Today, though, thanks to climate change, nearly a quarter of the map is obsolete. Some zones have moved, and others have dwindled dramatically. High-elevation zones and some interior plateaus have already lost around half their habitat—and could shrink by more than 80 percent by 2100. Seeds of trees that once would have thrived in a particular area might today be unable to even grow there. Zones may morph into ecosystems that are fundamentally different from what existed before, although exactly how much change is required before an ecosystem is "fundamentally different" is unclear and controversial.

Whether a particular population can adapt to change depends in part on how quickly the organism reproduces. Each new generation represents a chance to acquire useful traits. So a pine beetle, which reproduces quickly, has a much better chance of adapting than a tree, which is long-lived and slow to reproduce. A single bug may witness no change at all during its life span. A tree, though, has a front-row seat to global warming.

A stand of forest is most at risk during its first 20 years of life. Once the trees are established, they become far more resilient, "able to stick around for a while," says Brad St. Clair, a research geneticist at the U.S. Forest Service in Corvallis, Ore. But in the era of global warming, local conditions can change considerably over those two crucial decades.

"If you're going to move things to higher elevations so they'd be adapted to future climates," St. Clair says, "then they have to be adapted to cold-hardiness now." In other words, if you move warm-adapted trees now into a zone that is projected to warm in the future, those trees could be in short-term trouble because the zone is still cold today.

"We've got a moving target," Aitken admits. "Do we want to best match trees with climate when they're seedlings? Or 10 years

Maps by Andreas Hamann

© 2019 Scientific American

old? Or 30 years old?" One way to manage risk is to increase diversity—which might mean mixing local and nonlocal seeds. "You don't want to do the same thing on every hectare of ground. You can't plan around a single climate change scenario."

Assisted gene flow may be a good way to bulk up a forest's genetic diversity, sprinkling its gene pool with the ingredients that give trees a boost. As the environment shifts, some trees may suffer in the short term, but other trees will have genetic material that could help the forest weather tough times. "As those individuals that are more fit reproduce more," Aitken says, "we expect populations to start expanding again." The critical element, she says, is maintaining enough healthy trees to mate and survive while the process of adaptation unfolds.

Aitken, an avid backpacker and backcountry skier who owns a cabin in the woods of central British Columbia, hopes her work will help set new, smarter forest policy. She believes that if we do not begin practicing assisted gene flow, tree populations may begin to fail at the far northern or southern edges of species' ranges. "Trees might persist a long time, but they might stop reproducing," she says. "They'd be evolutionarily toast." They would become, she adds, a "land of the living dead." What's worse, they would hog space and sunlight that seedlings desperately need. Closer to the middle of a range, things would be a bit less dramatic. But trees might still grow more slowly or have trouble surviving. "Does that mean the populations there are just going to die?" Aitken asks. "Probably not. There's a lot of variation within populations. The species aren't going to go extinct, but I imagine you'd have pretty unhealthy-looking forests in the meantime." The poor health would harm other plants and animals because trees anchor entire ecosystems, providing food and shelter, regulating water flow and preventing soil erosion.

Around the world, Aitken says, "there has been very little attention paid to the movement of individuals within existing species ranges." The ecological risks are lower than transplanting truly foreign trees because such foreigners are not already part of the ecosystem, even though they may possess some desirable traits.

There are still risks to a gene-flow approach. It could, for instance, add local gene variants that would actually harm a larger population's chance for survival. "There is a risk that you could be introducing unwanted alleles," says Andrew Weeks, a geneticist at the University of Melbourne. But the problem would likely correct itself, he adds. "That's the beauty of natural selection, which will weed out these variants. By increasing the gene pool, you are giving the population the best chance for the future."

With British Columbia's forests worth $10 billion a year—as well as providing vital services such as preventing floods and soil erosion—doing nothing may pose an even greater risk. British Columbia has already seen what global warming can do to forests. Since the mid-1990s beetle invasions and wildfires, both linked to warming temperatures, have destroyed millions of acres of forest and consumed hundreds of homes. "We've had lots of wake-up calls here in terms of climate change," says Gregory O'Neill, a research scientist at British Columbia's Ministry of Forests and Range. The insects and fires, O'Neill says, left people in the province "quite cognizant of climate change—and not that it's some abstract thing in the future, but it's already happening."

SEEDS OF CHANGE: The AdapTree experiment gathers seeds from different habitats (*1*). Pine seedlings grown in the project's greenhouse (*2*) show variation in shape (*3*). Some needles are tested with a probe (*black rod*) to see whether they can resist freezing temperatures (*4*).

The losses jolted the provincial government into action. In 2009 British Columbia began revising its rules on moving seed. That same year O'Neill began an assisted migration trial for the province's forests, hoping to determine whether, where and how foresters might plant completely different species after harvesting. At 48 sites throughout Canada and the western U.S.—from Whitehorse to Sacramento—researchers planted 15 species of commercially important trees, moving them from their home range and, in some cases, relocating them thousands of miles away.

The extreme migration, O'Neill says, is merely a research tool, a way to provide a better overall picture of how the trees will fare. It is not intended as a guide for long-distance moves. Any actual changes in planting patterns will be incremental. "Something like

JACK WOODS *AdapTree project, University of British Columbia (1);*
CONNOR FITZPATRICK *AdapTree project, University of British Columbia (2)*

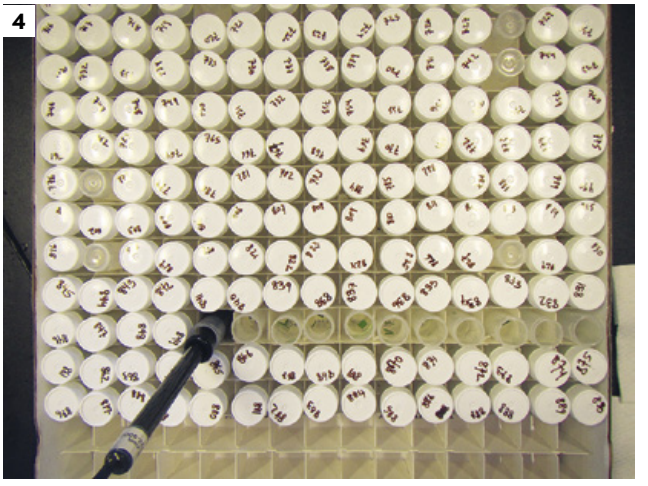
© 2019 Scientific American

'Do not move your trees downhill' or 'Do not move your trees south,' " he says. There is a weather station at each site, and the study will show how the growth and survival of the seedlings relate to the local conditions. Then, O'Neill says, scientists will be able to predict how the trees will respond to climate change.

The genetic analysis of AdapTree offers a complementary way to predict how the trees will fare. Researchers on the sprawling project pored over DNA sequences from millions of locations in the genomes of interior spruce and lodgepole pine trees. They developed a quick screening method—similar to that used by the human genome screening business 23 And Me—that looks at roughly 50,000 short strings of genetic code, known as single nucleotide polymorphisms, or SNPs. With that done, they dug in, as Aitken put it, trying to zero in on the specific polymorphisms that match a tree to its home base.

The raw genetic data from AdapTree are dizzying. Printed on both sides of sheets of 8 ½ by 11 paper, Aitken notes, the stack would rise at least 150 kilometers. And that is only part of the information. Researchers are now looking at how the genes actually function—how their instructions are carried out—when the trees encounter stresses such as drought or high temperatures.

3



4

JACK WOODS *AdapTree project, University of British Columbia (3);* PIA SMETS *AdapTree project, University of British Columbia (4)*

FORESTS OF THE FUTURE

A FEW DEGREES of latitude south, specialists in the U.S. Forest Service are beginning to weigh the pros and cons of assisted gene flow. "Where we're at is a lot of talk and discussion," St. Clair says. In the U.S., foresters historically did not focus on specific climate variations within zones where they collected and planted seeds. Moving seeds in a zone did not appear to involve enough temperature change to affect plant health.

Now foresters generally agree they need to get much better at moving seed. For as long as people have been planting trees, we have been relocating seed across rivers, villages, continents or oceans. "If you go back far enough, people used to move seed around all the time, and you'd often end up with failed plantations because people had no idea what they were doing," says Glenn Howe, a forest geneticist at Oregon State University. Partly as a result of those failures, over time the forestry community developed an aversion to risk. In the western U.S., seed zones, which guide how far seeds can be moved for planting, are narrow and conservative. "That's probably appropriate in a static climate," Howe says. "But with climate change, a very conservative approach could be a problem."

British Columbia is forging ahead. The province recently released new guidelines for reforestation, based in part on AdapTree's findings. Roughly 300 million seedlings will be planted to regrow commercial forests in B.C. this year. As of April 2018, the guidelines were voluntary, to give companies time to shift their seed inventory, but within a couple of years they will be the rule.

Of course, broader challenges to assisted gene flow remain. For the moment, researchers will need to convince resource managers to trust in genomic data, something they cannot see for themselves in the field. Aitken says viewpoints are definitely shifting, particularly surrounding the idea of moving things around within a species' range. "I wont say there's no resistance, but there's not a lot of resistance," she says. Still, it is crucial that all those nucleotide polymorphisms and sequence data "translate into a forester's lexicon," Aitken says.

Because ultimately all those strands of DNA make up living, breathing trees—the ones we depend on to construct our built environments as well as our natural ones. To thrive in a changing world, some of those trees may need to branch out into new territory. And to do that, they are going to need our help. SA

Hillary Rosner is a freelance writer based in Colorado. She has written for *National Geographic*, the *New York Times* and *Wired*, among other publications.

MORE TO EXPLORE

Placing Forestry in the Assisted Migration Debate. John H. Pedlar et al. in *BioScience*, Vol. 62, No. 9, pages 835–842; September 2012.

Assisted Gene Flow to Facilitate Local Adaptation to Climate Change. Sally N. Aitken and Michael C. Whitlock in *Annual Review of Ecology, Evolution, and Systematics*, Vol. 44, pages 367–388; November 2013.

Evaluation of Demographic History and Neutral Parameterization on the Performance of F_{ST} Outlier Tests. Katie E. Lotterhos and Michael C. Whitlock in *Molecular Ecology*, Vol. 23, No. 9, pages 2178–2192; May 2014.

CoAdapTree project: **http://coadaptree.forestry.ubc.ca/about**

scientificamerican.com/magazine/sa

© 2019 Scientific American

Can We Save the

Scientists are urgently transplanting, fertilizing and enhancing corals to help them adapt to warmer oceans, but rebuilding entire reefs will be daunting

By Rebecca Albright

ACROPORA CORAL in the Great Barrier Reef releases bundles of sperm and eggs. Corals along the reef's thousands of kilometers spawn once a year, during late summer.

© 2019 Scientific American

Corals?

© 2019 Scientific American

I'M STANDING ON A BEACH IN AUSTRALIA, TOES DIGGING INTO THE SAND, ZIPPING UP MY wet suit before I dive down to the Great Barrier Reef. As I stare out at the ocean, I'm excited by memories of my previous dive at this site a decade earlier. Growing up in Ohio, I had spent my childhood reading *A Day in the Life of a Marine Biologist* when I wasn't glued to the Discovery Channel. I got certified for scuba diving in one of Ohio's murky limestone quarries and made it to the Great Barrier Reef a year later. I'm remembering the anticipation squeezing my chest the day of that dive. My friend Emily, now an expert in marine algae, and I took bets on how long we could make our air last, which turned out to be about two magical hours. We were mesmerized by a forest of vibrant corals teaming with cuttlefish, giant purple clams and graceful sea turtles.

Now I am back, this time as a postdoctoral researcher at the Australian Institute of Marine Science. I wade in up to my chin, tip my head underwater and look through my mask. My heart drops. Gone are the cuttlefish. Gone are the giant clams. Gone are the turtles. The corals are drab. Most of the thriving life has been replaced by algae and sediment. Although I know senior scientists who shared gut-wrenching stories of how a particular reef had degraded over their long careers, I feel I am too young—barely 10 years in—to see this alarming degree of change. Shouldn't I be having this experience at the end of my tenure, not the beginning? Or better yet, not at all?

DIVERS secure new coral fragments raised onshore at Florida's Mote Marine Laboratory back onto a reef so they will grow and fill it in, a strategy similar to reforestation on land.

My shocked realization happened in 2014, as the third global mass-bleaching event began. Corals, often mistaken for rocks, are made of living animal tissue that contains microscopic algae, which provide the organism with food and give it color. When rising ocean temperature stresses corals, they expel the algae, causing the tissue to bleach—turn white—and leaving it vulnerable to starvation and disease. The mass bleaching persisted for three years, ruining reefs and breaking hearts worldwide. Although coral reefs can be threatened by overfishing, pollution and ocean acidification, the rapid and widespread destruction from warming is the greatest concern today.

The first major global bleaching events hit in 1998 and 2010, each time triggered by warming seas worsened by El Niño conditions. The 2014–2017 event was by far the longest and most extensive, harming more than 70 percent of the world's coral reefs. Two thirds of the Great Barrier Reef were reported as dead or severely bleached, and the devastating effects continue. Reefs are disappearing before our eyes. In the past 30 years we have lost around 50 percent of corals globally, and a recent report released by the United Nations's Intergovernmental Panel on Climate Change (IPCC) estimates we're at risk of losing more than 90 percent of coral reefs by 2050. We need solutions, and we need them fast.

Although reefs cover just 0.1 percent of the ocean floor, they support nearly 25 percent of all marine species, including fisheries that feed millions of people worldwide. They also provide natural breakwaters that protect coastal communities by reducing wave energy by up to 97 percent and wave height by up to 84 percent. And they generate vast tourism revenue. If we lose reefs, we jeopardize the livelihoods of 500 million people

IN BRIEF

Ocean warming is killing corals. Scientists are trying several approaches to help them cope, including transplanting lab-fertilized corals into the wild.

Researchers have found that stressing corals can turn on genes that lead to more resilient offspring, and enhancing certain algae can boost coral health.

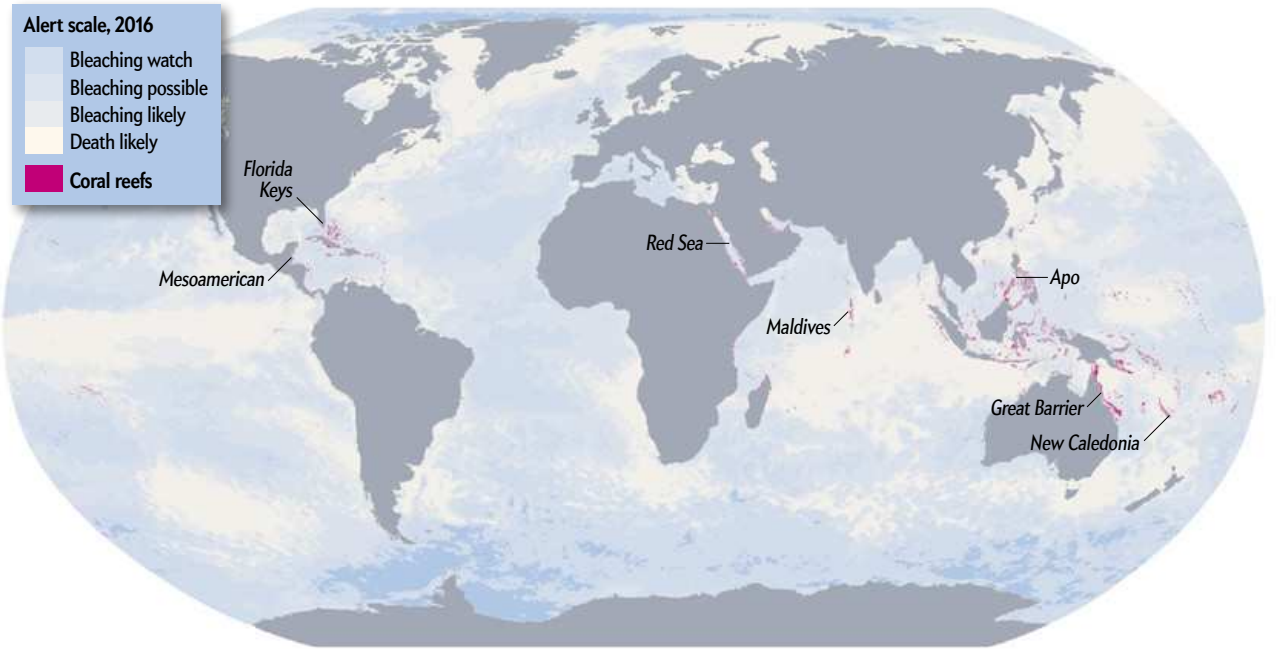
These techniques could restore reefs on a regional scale, but a worldwide revival can occur only if humans slow global warming.

PRECEDING PAGES: GETTY IMAGES; THIS PAGE: JOE BERG *Way Down Video*

© 2019 Scientific American

Worst Bleaching on Record

Relentlessly warm ocean water from 2014 to 2017 created the most extensive coral bleaching ever recorded. More than 70 percent of the earth's coral reefs were harmed. In 2016 alone (*map*), severe conditions spanned the globe (*white regions*). Hot water stresses corals, causing them to force algae out of their tissue, cutting off their food supply and leading to emaciation or death.



SOURCE: NOAA SATELLITE AND INFORMATION SERVICE

and more than $30 billion annually in goods and services. Even if you do not directly benefit from coral reefs, their destruction touches a chord in many people. As my colleague Luiz Rocha of the California Academy of Sciences puts it, "I may never live to see the *Mona Lisa,* but I still wouldn't want it to burn."

Driven by urgency, scientists are trying increasingly bold and creative ways to conserve and restore reef ecosystems. We are looking for techniques that are scalable and will not break the bank. Right now we are focusing on a handful of options that build on one another and can be integrated, including natural processes and human assistance. Together the steps might give corals the chance they need to make it through the coming decades, after which, it is hoped, the world will have drastically reduced its emissions, so warming will slow down.

I'm frequently asked: Will coral reefs survive? I think the answer is that they are resilient and might be able to cope, but they need breathing room—now.

NURSING CORALS BACK TO HEALTH

IF YOU WERE TO DIVE some seven kilometers off the coast of Florida, you might happen on one of several underwater forests of plastic trees with corals suspended from branches, like ornaments decorating a Christmas tree. Researchers are using such nurseries, as well as ones on land, to grow corals that can then be transplanted, or "outplanted," onto degraded reefs. Nurseries take advantage of the fact that all corals can reproduce sexually and asexually. Corals are clonal organisms—animals that are made up of hundreds to thousands of genetically identical polyps that are all clones of one another. They can reproduce sexually by creating eggs and sperm that fuse to create larvae and asexually when one polyp buds another.

When a coral is damaged by a storm, a piece of a colony might break off, tumble away, and eventually reattach to the bottom and continue to grow by cloning itself. Nursery practitioners can therefore deliberately fragment corals to create genetically identical clones. Today almost 90 species are successfully farmed around the world. Practitioners in the Caribbean and western Atlantic now grow and outplant tens of thousands of corals onto degraded reefs every year, often funded by private donors, grants or government restoration projects.

Scientists are looking to ramp up this restoration. About 13 years ago Dave Vaughan worked at Mote Coral Restoration. The techniques the researchers relied on then produced 600 corals in six years. The price tag on a single coral was about $1,000. But he discovered that because of a natural healing response, corals that are broken into tiny, eraser-size "micro fragments" can grow 25 to 50 times more quickly than corals in the wild. If pieces with the same genetic makeup (from the same parent) are placed a few centimeters apart, they will reconnect into a larger colony.

Now Erinn Muller, science director of Mote, says that the team can grow those 600 corals in an afternoon and has succeeded with all the half a dozen species it has tried; they are operating at less than $100 per coral, with some species costing only $25 per coral. By integrating citizen scientists and volunteers, Mote is convinced it can drop well below that number. The team can

Map by Mapping Specialists

© 2019 Scientific American

grow football-size corals that would have taken years to grow in the wild. Mote intends to produce and outplant up to 20,000 corals this year and grow this effort in years to come. Although the U.S. National Marine Fisheries Service says recovering the endangered Caribbean staghorn and elkhorn corals will require a minimum of $255 million and 400 years, Vaughan, who now works at Plant a Million Corals in Florida, hopes to remove them from the endangered species list in his lifetime.

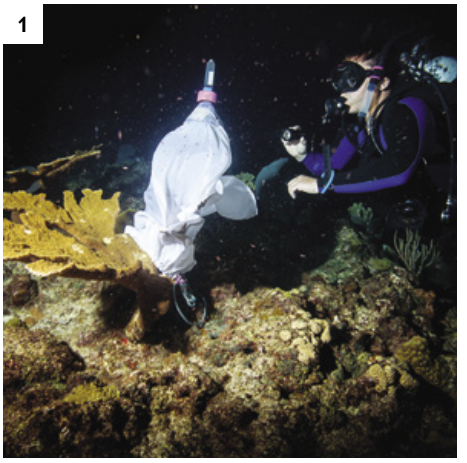
We're now very good at growing corals and can, in many cases, successfully restore reefs to their historical range and function—at the local level. But a jump to the ecosystem level is massive. One of the toughest challenges is how to meaningfully scale restoration efforts to the big leagues. Most efforts cover less than a hectare, whereas reef degradation is occurring over hundreds to thousands of square kilometers. The price tag to replant the extensive Great Barrier Reef system, 2,300 kilometers in length, has been estimated at nearly $200 billion, using fragments at $5 apiece. But even that cost could be well worth it because the recovery would restore large fisheries that feed many people, create many jobs, and protect valuable coastlines and communities from storms.

CORAL SEX

ALONG WITH GROWING corals asexually in nurseries, scientists are having increasing success in helping corals reproduce sexually, which can broaden genetic variation. As populations decline, genetic diversity is lost, which lessens corals' ability to resist warming water. Many Caribbean reefs, for example, are dominated by a single clone, and both science and history teach us that relying on low genetic variation, particularly in times of environmental change, can lead to disaster. In the 1800s, for example, a single clone of the Irish lumper potato fed Ireland's growing population until a rot wiped it out, devastating the island's people and economy. A more diverse crop would have fared much better. As in potatoes and people, genetic diversity can make corals less susceptible to environmental stress.

Sexual reproduction is nature's way of building diversity. Corals are fixed to the ocean floor, so they cannot move around in search of a mate. To reproduce sexually, most corals release eggs and sperm into the water column where, fingers crossed, fertilization happens. In degraded areas, where corals are few and far between, this becomes increasingly unlikely.

At the California Academy of Sciences, we are partnering with The Nature Conservancy and Secore International, a coral conservation organization, to help shepherd corals through this tricky process. We now have a good idea of when different coral species spawn. On predicted nights of spawning—after sunset, near a full moon in late summer (corals are surprisingly romantic)—coral colonies release eggs and sperm. We descend into the water with nets to harvest them and transfer these gametes to the laboratory, where we fertilize them in buckets of seawater. The resulting larvae are generally the size of sesame seeds and are vulnerable to being eaten in the wild until they settle and start to grow, so we raise them until they are big and healthy enough to be outplanted onto the reef. The goal is not to replant entire reefs but to maximize genetic variation and to rebuild enough of the population so the reef can recover naturally and be more resilient to the environment.



ELKHORN CORAL'S sperm and eggs are collected underwater (*1*) in a tube (*2*). In the lab, researchers combine the gametes with those from other corals to make new varieties, which increases genetic diversity, improving resilience to ocean stresses.

PAUL SELVAGGIO *Secore International*

Many reefs have low genetic diversity, preventing them from churning out coral babies. By combining asexual and sexual restoration techniques, we may be able to restore one reef to the point where it can rebuild healthy reefs nearby. The aim is to create something that has a life beyond itself.

In the wild, only about one in a million coral babies survive. We are doing everything we can to help them through the vulnerable early stages. We can now achieve almost 100 percent success in fertilizing corals in the lab and settling the larvae onto tiles that can be outplanted, increasing the number of sexually compatible individuals that can improve future reproduction without our help. At a spawning event in Curaçao in the Caribbean in 2017, I helped a team collect five million eggs from 25 colonies within two short days. This is a new record for SECORE "and shows the scales we could work with," says the organization's founder Dirk Petersen.

In addition to helping corals reproduce in the wild, a handful of groups have started trying to spawn corals in public aquaria. No small feat, as it depends on our ability to mimic the suite of environmental cues that trigger reproduction, including lunar and seasonal cycles. Luckily, technological advances in aquaria-control systems are making it possible. At the California Academy of Sciences, I lead a team who have achieved two successful coral-spawning events—an accomplishment achieved in only one other location worldwide, the Horniman Museum in London. Our goal is to use this system to better understand the fundamental biology underlying coral reproduction and develop and validate methods that will help scale restoration efforts in the wild.

One of the biggest hurdles is keeping baby corals alive once we

© 2019 Scientific American

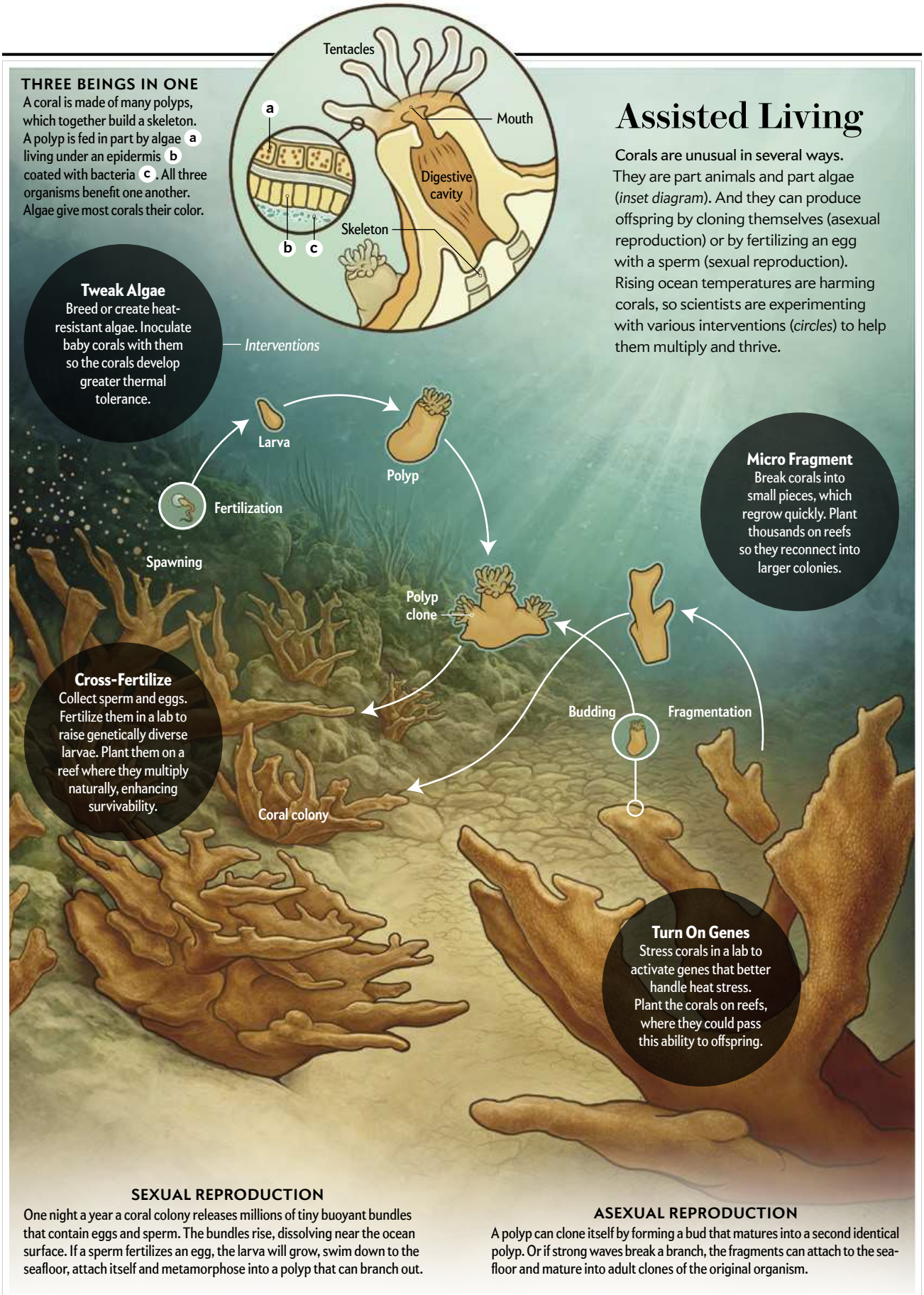

THREE BEINGS IN ONE
A coral is made of many polyps, which together build a skeleton. A polyp is fed in part by algae a living under an epidermis b coated with bacteria c. All three organisms benefit one another. Algae give most corals their color.
Tentacles
Mouth
Digestive cavity
Skeleton
a
b
c
Assisted Living
Corals are unusual in several ways. They are part animals and part algae (*inset diagram*). And they can produce offspring by cloning themselves (asexual reproduction) or by fertilizing an egg with a sperm (sexual reproduction). Rising ocean temperatures are harming corals, so scientists are experimenting with various interventions (*circles*) to help them multiply and thrive.
Tweak Algae
Breed or create heat-resistant algae. Inoculate baby corals with them so the corals develop greater thermal tolerance.
Interventions
Larva
Polyp
Fertilization
Spawning
Micro Fragment
Break corals into small pieces, which regrow quickly. Plant thousands on reefs so they reconnect into larger colonies.
Polyp clone
Cross-Fertilize
Collect sperm and eggs. Fertilize them in a lab to raise genetically diverse larvae. Plant them on a reef where they multiply naturally, enhancing survivability.
Budding
Fragmentation
Coral colony
Turn On Genes
Stress corals in a lab to activate genes that better handle heat stress. Plant the corals on reefs, where they could pass this ability to offspring.
SEXUAL REPRODUCTION
One night a year a coral colony releases millions of tiny buoyant bundles that contain eggs and sperm. The bundles rise, dissolving near the ocean surface. If a sperm fertilizes an egg, the larva will grow, swim down to the seafloor, attach itself and metamorphose into a polyp that can branch out.
ASEXUAL REPRODUCTION
A polyp can clone itself by forming a bud that matures into a second identical polyp. Or if strong waves break a branch, the fragments can attach to the seafloor and mature into adult clones of the original organism.

Illustration by Rebecca Konte

© 2019 Scientific American

place them back in the sea. After all, degrading conditions are the main cause of coral decline in the first place, so until we tackle climate change, pollution and overfishing—via policy, awareness and global changes to the way we live—we are basically using a Band-Aid approach to buy reefs more time to try to survive. Along the way, we may be lucky enough to create genotypes through sexual reproduction that we could mass-produce with asexual techniques. We could then outplant them so Mother Nature could select which species might thrive.

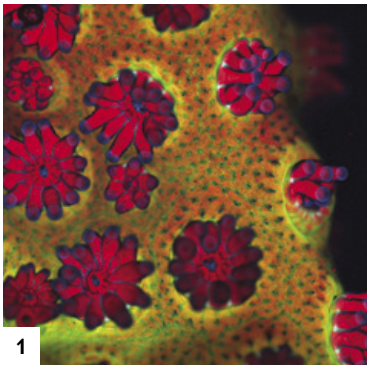
SUPERCORALS

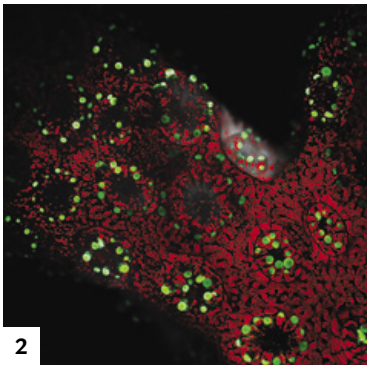
WE CAN'T DEPEND ON good fortune for that scenario to happen. And because the ocean environment is changing too rapidly for many corals to adapt naturally, scientists are exploring other ways to accelerate the pace of adaptation. One approach is human-assisted evolution—enhancing traits that boost the capacity of corals, among other reef organisms, to tolerate stress and recover after bleaching events. Human-assisted evolution already surrounds our everyday lives. Most of the food we buy has been selectively bred or modified in some way (think "disease-resistant tomatoes"). Our pets have been selectively bred for certain aesthetics and personality traits. So why not breed or enhance corals to resist climate change?

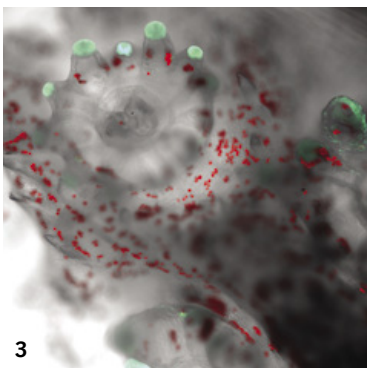
Madeleine van Oppen of the Australian Institute of Marine Science and the Gates Coral Lab, directed by the late Ruth Gates, of the Hawaii Institute of Marine Biology are collaborating to enhance stress resistance. The Gates lab put corals on "environmental treadmills" to condition them to handle stress. Exposing certain corals in the lab to sublethal temperature stress may actually prompt them to turn on certain genes that help them better handle greater thermal stress in the long run. This process, known as epigenetic tuning, might be even more exciting if the trained corals are transplanted onto reefs, where they can transfer this ability to offspring, creating a generation of "supercorals." In theory, epigenetic tuning could enhance corals' ability to resist bleaching.

We are only beginning to understand this process. Early lab tests are promising, but field trials have yet to be conducted. Once done, they will reveal whether transplanted corals confer enhanced abilities to subsequent generations, whether the approach can be scaled up and at what cost, and whether any inherent risks exist.

Van Oppen is exploring selective breeding. A certain amount of genetic diversity exists within each species, leaving some of them more or less resistant to bleaching or disease. As breeders do with pets to optimize desired traits, if we can identify resistant coral colonies and breed them to produce resistant offspring, we may be able to improve the temperature tolerance of an entire reef as subsequent generations pass down the useful genes.







ALGAE (*red dots*) **feed a healthy** ***Pocillopora*** **coral (*1*). Warming seas drive them off (*2*) until the coral is bleached (3), starving it of sugars. (Green dots are proteins.) Scientists hope to devise heat-tolerant algae that would persist.**

Breeding corals is difficult because they can take up to a decade to fully mature. Adapting to environmental change is hard for the same reason. But microbes and algae that live in symbiosis with corals typically mature rapidly and can influence a coral's health tremendously. We are thus trying to manipulate these organisms via artificial selection in ways that boost coral health. In recent years scientists have realized just how much our microbiome (the bacterial communities inside our body) influences our health, for better, for worse. Probiotics are now available in everything from yogurt to kombucha tea and even chocolate, claiming to boost digestion, immune function and overall health.

Van Oppen is now developing strains of algae in the lab and inoculating baby corals with them to see whether they confer thermal tolerance. She and the Gates lab have also been attempting to see if epigenetic tuning, selective breeding and microbiome manipulations done on the same corals could possibly be even more effective in combination.

It is still early days for most of the techniques we are trying. But some evidence suggests that they could be combined for even greater success. This approach might look like the following: First, we would use sexual reproduction and assisted evolution to generate improved and new diversity among coral populations and to create individuals that have greater stress tolerance. Then we would mass-produce them in nurseries using asexual techniques and outplant them to reefs.

Could this happen soon? Not exactly. Some techniques, such as selective breeding, are immediately tractable, inexpensive and effective. But more work is needed to establish the viability and scalability of other techniques and to gauge the risks of unanticipated ecological consequences. It is possible, for example, that artificially enhanced organisms might possess novel traits that allow them to outcompete native populations rather than integrating with them, which would undermine the very goal of helping reefs thrive. It is also possible, however, that the risk of doing nothing could be far worse.

FROZEN FOR THE FUTURE

WHETHER WE BOOST CORALS using single or combined techniques, one other step is vital: preserving sperm, eggs, larvae and entire coral fragments in the equivalent of seed banks,

AMY EGGERS (*1*); AMY EGGERS, KATIE BAROTT AND MINDY MIZOBE (*2,3*)

© 2019 Scientific American

RESEARCHER monitors transplanted corals in a bay surrounding the Hawaii Institute of Marine Biology to see how they are affected by acidic ocean water, another stressor imposed by climate change.

which agronomists have used for decades to help raise crop yields, disease resistance and drought tolerance. The banks allow researchers to pull out biological bits and pieces as needed to further improve resilience and diversity.

Taking a page from the in vitro fertilization (IVF) handbook, Mary Hagedorn of the Smithsonian Conservation Biology Institute has established the first genome repository for endangered coral species. In IVF, sperm or eggs are cooled with liquid nitrogen to extremely low temperatures. The eggs can be thawed, fertilized in the lab and transferred to the uterus as embryos. Originally developed for humans, the cryopreservation concept has spread to help endangered species worldwide.

In 2004, some years after the first human baby generated from a cryopreserved egg was born, Hagedorn created the coral cryoconservation program. Her team has developed a freezing system for sperm that can be applied to a wide range of coral species. To date, the team has successfully banked 16 species from around the world (2 percent of the earth's estimated 800 species). Thawed sperm have fertilization rates comparable to fresh sperm, and the resulting embryos develop normally into healthy juveniles.

Hagedorn has distributed this germplasm, or living tissue, to cryobanks in various countries. Theoretically, it could remain frozen and alive for hundreds to thousands of years. The germ line cells could later be thawed and used in natural and captive breeding programs. For example, frozen sperm could fertilize eggs from places far beyond the sperm's natural range, introducing new genes into the coral gene pool. And of course, the banks can preserve species that may decline or disappear if reefs collapse.

Hagedorn hopes to cryopreserve eggs (in addition to sperm) and whole larvae by 2020 before moving on to entire micro fragments. She is also developing techniques to cryopreserve fish testes to help conserve reef fish biodiversity. Ultimately she envisions a future in which the germplasms of corals and other endangered reef organisms are deposited in highly secure facilities, making eggs, sperm and embryos available to broaden genetic diversity and rebuild reefs. "We have no idea what science will be able to do in 100 years," Hagedorn says.

Where do we go from here? Although some of these solutions may seem too unconventional by today's standards, we must invest in strategies for tomorrow. Many of these techniques have yet to be tested beyond the conceptual or lab stage, and questions remain about the scalability, costs and ecological consequences of manipulating reef systems. The consequences of doing nothing, however, threaten corals and the many species that rely on them.

What we know for sure is that there is no single fix to the problems plaguing coral reefs. Scientists are throwing everything they have at different options to buy reefs time. Although none of today's techniques is likely to salvage reefs at the global scale, many show promise on local or regional levels. The reefs of tomorrow may not resemble the reefs of today, but they can still provide important goods and services to ecosystems and to people. Climate change, pollution and overfishing are the larger challenges. We have to tackle them collectively to protect oceans overall and give coral reefs the breathing room they need to survive. SA

Rebecca Albright is a coral biologist and curator at the California Academy of Sciences. She focuses on how coral reef ecosystems cope with changing environmental conditions.

MORE TO EXPLORE

Global Warming and Recurrent Mass Bleaching of Corals. Terry P. Hughes et al. in *Nature*, Vol. 543, pages 373–377; March 16, 2017.

California Academy of Sciences' reef program: **www.calacademy.org/explore-science/hope-for-reefs**

Mote Marine Laboratory's micro fragment program: **https://mote.org/research/program/coral-reef-restoration**

Secore International's coral conservation program: **www.secore.org**

scientificamerican.com/magazine/sa

THEOCEANAGENCY.ORG

© 2019 Scientific American

Meat Grown from Stem Cells

Beef for dinner—without killing animals or the environment

By G. Owen Schaefer

IMAGINE BITING INTO A JUICY BURGER THAT WAS PRODUCED without killing animals. Meat grown in a laboratory from cultured cells is turning that vision into a reality. Several start-ups are developing lab-grown beef, pork, poultry and seafood—among them Mosa Meat, Memphis Meats, SuperMeat and Finless Foods. And the field is attracting millions in funding. In 2017, for instance, Memphis Meats took in $17 million from sources that included Bill Gates and agricultural company Cargill.

If widely adopted, lab-grown meat, also called clean meat, could eliminate much of the cruel, unethical treatment of animals raised for food. It could also reduce the considerable environmental costs of meat production; resources would be needed only to generate and sustain cultured cells, not an entire organism from birth.

The meat is made by first taking a muscle sample from an animal. Technicians collect stem cells from the tissue, multiply them dramatically and allow them to differentiate into primitive fibers that then bulk up to form muscle tissue. Mosa Meat says that one tissue sample from a cow can yield enough muscle tissue to make 80,000 quarter-pounders.

A number of the start-ups say they expect to have products for sale within the next few years. But clean meat will have to overcome a number of barriers if it is to be commercially viable.

Two are cost and taste. In 2013, when a burger made from lab-grown meat was presented to journalists, the patty cost more than $300,000 to produce and was overly dry (from too little fat). Expenses have since fallen. Memphis Meats reported this year that a quarter-pound of its ground beef costs about $600. Given this trend, clean meat could become competitive with traditional meat within several years. Careful attention to texture and judicious supplementing with other ingredients could address taste concerns.

To receive market approval, clean meat will have to be proved safe to eat. Although there is no reason to think that lab-produced meat would pose a health hazard, the FDA is only now beginning to consider how it should be regulated. Meanwhile traditional meat producers are pushing back, arguing that the lab-generated products are not meat at all and should not be labeled as such, and surveys show that the public has only tepid interest in eating meat from labs. Despite these challenges, the clean meat companies are forging ahead. If they can succeed in creating authentic-tasting products that are also affordable, clean meat could make our daily eating habits more ethical and environmentally sustainable. SA

G. Owen Schaefer is a research assistant professor in the Center for Biomedical Ethics at the Yong Loo Lin School of Medicine at the National University of Singapore. He studies the ethics of developing novel biotechnologies and has written on the ethics of human enhancement, genetics, big data, assisted reproduction and in vitro meat.

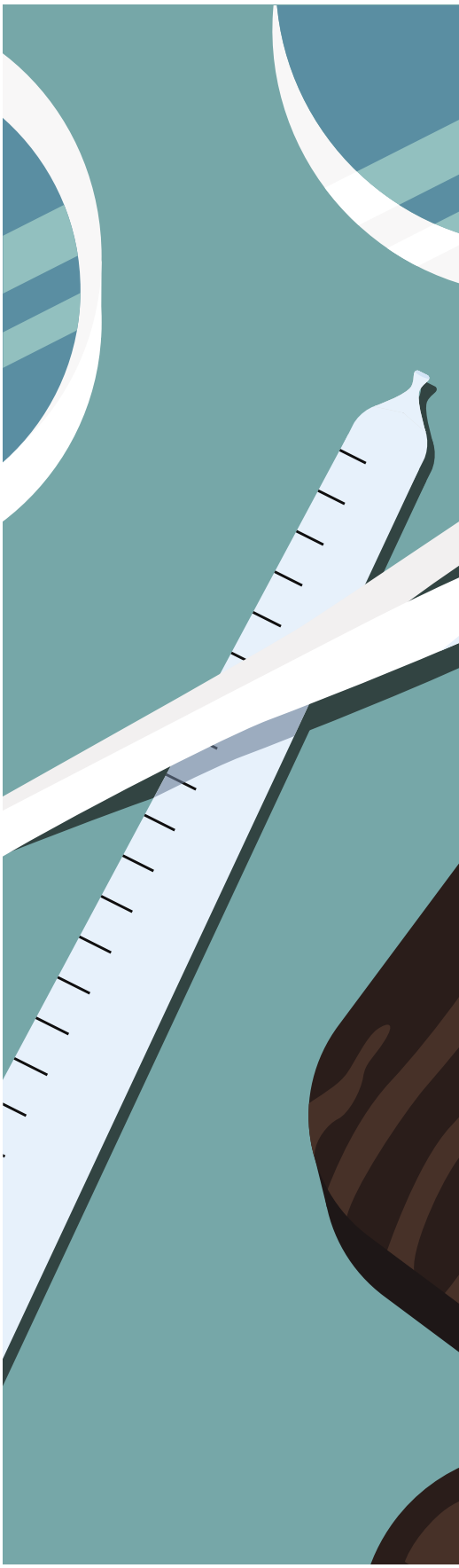


Illustration by Vanessa Branchi

© 2019 Scientific American

© 2019 Scientific American

COULD GENETIC ENGINEERING SAVE THE GALÁPAGOS?

In the Galápagos, invasive species are driving native animals to extinction. Some conservationists are asking whether genetic manipulation is the solution

By Stephen S. Hall

© 2019 Scientific American

MARINE IGUANAS of the Galápagos are vulnerable to feral cats and other invasive predators.

© 2019 Scientific American

ON SEPTEMBER 25, 1835, DURING THE HMS *BEAGLE*'S SOJOURN TO THE Galápagos archipelago, Charles Darwin first set foot on what was then known as Charles Island. He found a colony of 200 to 300 inhabitants, nearly all political exiles sent there by Ecuador, aka the "Republic of the Equator," after a failed coup attempt. The lowlands did not much impress Darwin, with their "leafless thickets," but after trudging four miles inland and upward to a small, impoverished settlement in the highlands, he found "a green and thriving vegetation," cultivated with bananas and sweet potatoes, along with a group of islanders who, "although complaining of poverty, obtain, without much trouble, the means of subsistence." That was mainly because of the tens of thousands of giant tortoises that once prowled these islands. "In the woods," Darwin noted, almost as an afterthought, "there are many wild pigs and goats."

On the morning of August 25, 2017, Karl Campbell bounded off a twin-engine motorboat and onto the dock of that same humble island. Now known as Floreana, the island has 144 residents, half as many as in Darwin's time, and Campbell seemed to know them all. Dressed down in a baseball cap, blue jeans and gray T-shirt that read "Island Conservation," he ambled up to Claudio Cruz, at the wheel of a local bus (a converted truck with benches in the back), and exchanged some banter. He waved hello to Juanita and Joselito, who manned the Ecuadorian government's biosecurity checkpoint on the dock. He shouted out another "*Hola*" to the postmaster, popped his head into the community center to greet Myra and Holger, a farmer, and paused to catch up with Carmen, the woman who monitors the public bathrooms near the landing. His path up Floreana's one paved road was interrupted by salutations, chitchat, short jokes and the one-cheek kisses that are the custom in Ecuador.

Campbell, 42 years old at the time of the interview, is an Australian who had lived in the Galápagos Islands for 20 years; he's a gregarious and outgoing fellow, with a tendency to begin conversations with "All good, mate?" But the cheery demeanor and bonhomie he displayed that morning is an essential part of a massive scientific undertaking. Campbell has a Ph.D. in vertebrate pest management from the University of Queensland in Australia, and in 2006 he began working as an animal removal specialist for Island Conservation, an organization based in Santa Cruz, Calif., that is devoted to preserving biodiversity and preventing extinctions by removing invasive species from islands throughout the world. Campbell has been working on eradications in the Galápagos since 1997, including a 2006 campaign to remove all the feral goats and donkeys from Floreana. A decade later he's a project manager with Island Conservation, and the most ambitious project on its agenda is once again on Floreana: to eradicate every single rat and mouse on the island.

There are hundreds of thousands of islands in the world. "You can't work on all of them," Campbell says. Conservationists, according to Campbell, "are currently able to do 10 to 20 islands a year to rid them of mice. So which are the ones you should be working on most urgently? We basically draw up a list of places where we should be working to prevent extinctions." Topping that list, he says, is Floreana.

"Floreana has one of the highest endemicity rates in the Galápagos, the highest rate of extinctions due to the invasive species here and the highest rate—by far—of critically endangered species, which makes it one of the highest-priority targets not just in the Galápagos but in the world," Campbell says, in a spiel that has the polish and urgency of countless recitations to funders, journalists and probably every one of Floreana's residents.

Floreana is at the limit of feasible projects using current eradication tools. The island is large (17,253 hectares, or about 46,600 acres), and it is inhabited, which complicates the task enormously. It means having to explain the logistics and consequences of the entire project—not least of which is a plan to

IN BRIEF

Invasive species have been a problem in the Galápagos Islands since mariners first arrived there. Hundreds of introduced species of plants, insects, reptiles, birds and mammals live in the archipelago, displacing and in some cases preying on native species.

Eradicating invasive species can be a brutal job. On the island of Floreana, a plan to eliminate the rodents that raid the nests of native birds and reptiles calls for 400 tons of rat poison, requiring weeks of dislocation for pets, livestock and perhaps children.

Genetic manipulation—for example, tweaking sex inheritance in rodents to produce an all-male, and thus reproductively doomed, population—is being discussed as a safer alternative to poison and bullets. But what are the risks? And would it even work?

PRECEDING PAGES: TUI DE ROY

© 2019 Scientific American

BROWN RATS (*1*) **are a primary target of a massive invasive-species eradication effort planned for the island of Floreana, where donkeys** (*2*)**, cattle** (*3*) **and many other nonnative species have been introduced over the centuries. On neighboring Isabela Island, feral goats denuded the landscape of a giant Galápagos tortoise stronghold** (*4*)**.**

dump 400 tons of rodent poison all over the island. That is why, since 2012, Campbell and his colleagues, such as Carolina Torres and Gloria Salvador, have been visiting Floreana almost once a month, enduring the bumpy two-hour boat ride from the main island of Santa Cruz to meet with residents, describe their proposed project, and figure out the massively complicated steps needed to protect adults, children, livestock, water and endangered species from the effects of the poison.

Such eradications require almost military-scale logistics and precision, which is why Campbell has been desperately seeking an alternative to the blunt-force tools of current techniques. One of the most appealing, to his mind, is a controversial new form of genetic manipulation known as gene drive. Compared with the frustrations he endures every day on the Floreana project, he likens the technology to a magic wand out of *Harry Potter*.

The basic strategy of using gene drive in the conservation setting would be to tinker with the DNA of mice, using either the new gene-editing tool CRISPR or other tools of genetic manipulation, in such a way as to tilt the odds of sex inheritance; one example would be to produce offspring that would be exclusively male, eventually producing a daughterless population of mice. The elimination of females, of course, would create a reproductive dead end for that invasive species. Gene drive is far from a practical technology at this point, but Island Conservation has been working with molecular biologists in the U.S. and Australia to create these genetically modified mice, and Campbell has made no secret of his enthusiasm for the approach at recent scientific meetings. And that, in turn, may be why the National Academies of Sciences, Engineering, and Medicine, in a 2016 analysis on the potential benefits and risks of gene drive, included the example of daughterless mice among a series of potential scenarios where the technology might be applied. As the report noted, "Perspectives on the place of human beings in ecosystems and their larger relationship to nature—and their impact on and manipulation of ecosystems—have an important role in the emerging debate about gene drives." That debate, in a sense, has already begun on Floreana, where residents have been weighing the benefits and risks of a massive, albeit nongenetic manipulation of their precious ecosystem for the past five years.

Campbell is the first to acknowledge that the Galápagos will not be the first or best place to test gene drive in the field. But it may be the best place to think about the implications, good and bad, of gene drive in the context of species preservation. If, as a global community, we value the preservation and protection of biodiversity in the Galápagos (a value ratified by its selection as among the first World Heritage sites by the United Nations agency UNESCO), we also have to come to terms with the complexities and paradoxes of invasive-species eradication, which legiti-

KRYSTYNA SZULECKA *Alamy (1, 3)*; WOLFGANG KAEHLER *Getty Images (2)*; TUI DE ROY *(4)*

© 2019 Scientific American

mizes the local elimination of certain animals for the benefit of other species—not least humans. As Campbell likes to point out, "No one comes to the Galápagos to see rats and goats and cats."

1. "RED IN TOOTH AND CLAW"

IF THE GALÁPAGOS ISLANDS have become synonymous in the public imagination with ecological harmony and thrillingly pristine biodiversity, the reality is somewhat different. Yes, the massive tortoises are stunning, but where thousands of them once bulldozed the highlands of Floreana, there are now about two dozen—all imported from other islands because the local species went extinct. Yes, the fearless finches are charming and beautiful, but the Floreana mockingbird disappeared from the island around 1880, one of 13 species that have gone locally extinct. Yes, the sea turtles languorously swimming off La Lobería Beach are magnificent, but their eggs have been relentlessly poached by indifferent predators. All those iconic Galápagos species have been ruthlessly threatened by invasive species.

There is a darkness to the Galápagos paradise, and it has been there a long time, perhaps since Tomás de Berlanga, then the bishop of Panama, went off course and discovered the islands in 1535. The first true invasive mammals on the islands were the pirates who frequented them in the 17th century, followed by sailors from whaling ships in the 18th century. These mariners brought in tow a malign ark of mammalian deplorables they introduced to islands that had been largely unperturbed for millions of years. If you want to be provocatively precise about it, the very first documented resident invasive species on Floreana was an Irish sailor named Patrick Watkins, marooned around 1805. He reportedly grew vegetables, which he bartered to visiting ships in exchange for rum (he was the model for a story by Herman Melville).

Three years before Darwin's arrival, a zoo's worth of invasive species had become entrenched on Floreana. It is no accident that in the scientific literature, the earliest date for many invasive species is 1832. That's when General José de Villamil, the first governor of the Galápagos Islands, arrived on Floreana to organize the penal colony. As Cruz—farmer, amateur historian, sometime bus driver and the largest landowner on Floreana—puts it, "He brought everything—goats, donkeys, cows, mules, horses, dogs, pigs, rats, everything." Similar animal importations occurred on other islands in the Galápagos during the 19th century, with devastating consequences on the local flora and fauna. Villamil brought the mules and donkeys to haul tortoises down from the highlands. At the time of his visit, Darwin reported that a previous ship visiting Floreana had loaded up on 200 tortoises in a single day (other ships reportedly collected as many as 700 apiece, according to Darwin).

Invasive mammals have wrought havoc on the ecosystem, in direct and indirect ways. Donkeys destroy tortoise eggs when they roll on the ground to cleanse themselves. Feral cats devour seabird chicks and snack on baby lava lizards, as do mice. Feral goats, in buzz cut fashion, chew through the native vegetation, removing the food that sustained the tortoise population for centuries and clearing the way for invasive plants such as guava, which has spread throughout the highlands. The Galápagos racer, once a common snake? Gone. More than 750 alien plant species and almost 500 alien insects have taken root in the Galápagos. As much as the islands have been a global classroom on evolution, they are also a reminder that nature is not static and that conservation sometimes alters nature to preserve it.

It has been the same story throughout the archipelago, though with some very odd chapters. In a 2012 compendium of "alien vertebrates" on the Galápagos, R. Brand Phillips, David A. Wiedenfeld and Howard L. Snell, all then affiliated with the Charles Darwin Research Station in Puerto Ayora on Santa Cruz, catalogued a rogue's gallery of 44 uninvited guest species, nearly half of them establishing feral populations. They ranged from obvious interlopers (goats, pigs, cattle, black rats) to an unwelcome menagerie of exotic animals. The Nile tilapia, a freshwater fish, turned up on the island of San Cristóbal in 2006; tree frogs have been spotted on two islands. Over the years nonnative visitors have included the mourning gecko, domestic ducks, cattle egrets, parakeets, peafowls and grackles. Three monkeys, of uncertain species, turned up on Floreana in the 1930s, and in 1937 one local entrepreneur tried to establish an ocelot colony on the island of Santiago. Ocelots!

Humans don't get a waiver from these waves of invasion, and their impact is increasing, too. In 1984 only 6,000 people total lived on five of the 129 islands and islets; more than 30,000 do today. And tourists? Three decades ago there

Unnatural Selection

Island Conservation, a California-based organization, is helping Ecuadorian authorities plan the eradication of every rat and mouse on Floreana, a large, inhabited island in the Galápagos archipelago.

Map by Mapping Specialists

© 2019 Scientific American

1

2

BLACK RATS (*1*), **which came to the Galápagos on ships as early as the 17th century, devour eggs laid by native reptiles and birds. To fight back, biologists have resorted to baiting the nesting areas of the Galápagos storm petrel** (*2*) **and other species with rat poison.**

were 20,000 a year; in 2016 there were 218,000. Just as more people began to come to the Galápagos to marvel at the local biodiversity, that biodiversity became increasingly threatened by the invasive species.

The Galápagos National Park Service, which controls 97 percent of the land in the archipelago, first attempted to eradicate goats on Pinta Island in 1971—an undermanned campaign that proved the adage in the eradication business that "a 99 percent success is a 100 percent failure." Only 10 goats remained on the island after the eradication program, recalls Victor Carrion, a former national park service official who participated in many eradication efforts. Within 10 years the number had climbed back up to 2,000. "The problem," Carrion says with a shrug, "was the final stage."

The Galápagos National Park Service began to develop more effective eradication plans in the late 1990s. Around this time, Campbell, then 22 years old and trying to decide what to do with his life, turned up in the archipelago. He had no particular affinity for the Galápagos—except, perhaps, that as a teenager back in Brisbane, he kept hundreds of pet birds in aviaries he built himself. In August 1997 he served as a volunteer on a goat-eradication project on the island of Isabela. Within a decade he would play a leading role in some of the most ambitious—and controversial—island eradication projects in the world.

II. THE DEVIL WE KNOW

ERADICATION IS AN UGLY, EUPHEMISTIC BUSINESS. In 2004 the national park service and the Charles Darwin Foundation initiated a more systematic campaign to eradicate goats from the northern, uninhabited part of Isabela, the largest island in the archipelago. Two helicopters were used for aerial hunting; two or three hunters in each helicopter shot goats from the air, using semiautomatic 12-gauge shotguns and semiautomatic .223-caliber AR15 rifles. After the first aerial sweep, ground hunters with specialized dogs went into heavily vegetated parts of the island to flush out goats that had survived the initial onslaught. In the final phase, beginning in March 2005, the eradication team deployed some 700 "Mata Hari goats" and "Judas goats."

Campbell's Ph.D. project was the development of the Mata Hari goat—a variation on the Judas goat, which was developed in the 1980s. Judas goats are outfitted with radiotelemetric collars. The animals are very gregarious, so hunters use goats wearing a wire, if you will, to find other goats. Mata Hari goats take the gambit one step further—they are female goats outfitted with hormonal implants that induce a permanent state of estrus, so that they seek and attract male goats. Mata Hari goats, needless to say, were not cooked up in the evolutionary hot pot of the Galápagos. Indeed, Campbell trained local hunters to perform field surgery on female goats—tying their fallopian tubes, terminating any pregnancies and inserting hormonal packs so that they were in constant heat, after which they were outfitted with radiotelemetry transmitters on collars so they could be traced. Once released, the Judas and Mata Hari goats tracked down the last holdouts. When all was said and done, Project Isabela killed 62,818 goats, at a cost of about $4.1 million. To hear Carrion tell it, the main complaint of the locals was that they didn't get any of the meat. "They said, 'We're hungry, and we need the food!'" he recalls. Even 100 percent success, in this case, wasn't enough—on at least nine occasions, according to Carrion, disgruntled locals deliberately reintroduced eradicated species, in part to protest local fishing regulations.

TUI DE ROY (*1*); PETE OXFORD *Getty Images* (*2*)

© 2019 Scientific American

FLOREANA GIANT TORTOISES were once thought extinct, but recent genetic research identified related species living on nearby Isabela. Biologists are breeding the tortoises and reintroducing them to Floreana.

But the magnitude of the eradication campaigns in the Galápagos is staggering: 79,579 goats "removed" from Santiago, 41,683 from Pinta, 7,726 in San Cristóbal—in all, 201,285 goats have been "removed" from 13 islands (and you know it's a grisly business when euphemisms such as "removed" are used instead of "killed"). It's a pretty good bet that the tourists who flock to the Galápagos to swim with the sea turtles and follow the graceful arc of its storied birds are unaware that the islands have been turned into killing fields over the past two decades to preserve their famous biodiversity.

Even a modest rodent-eradication campaign illustrates just how tricky the traditional approaches can be. In 2012 the Galápagos National Park Service and collaborators began applying the rodent poison brodifacoum on the small, uninhabited island of Pinzón to eliminate rats, which had ravaged the eggs and hatchlings of giant tortoises for decades. The eradication was successful, and substantial numbers of tortoise hatchlings were reported on the island for the first time in a century. But the poison made its way into lava lizards, which in turn were eaten by endangered Galápagos hawks, resulting in at least 22 deaths because of brodifacoum poisoning (even though many of the hawks had been protected by "captive holding" for two weeks). In one instance, researchers found extremely high levels of rat poison in an owl carcass more than two years after the baiting.

And that brings us to the most ambitious island eradication in the Galápagos and perhaps anywhere in the world, an endeavor that everyone on Floreana refers to simply as the "*Proyecto*"—the Project.

III. THE DEVIL IS IN THE DETAILS

THERE IS ONE STORE in Floreana and one main road. As elsewhere in the Galápagos, the houses are simple cinder block constructions with corrugated metal roofs. If you go to one of the few restaurants in the island's single town, you better tell them ahead of time that you are coming: otherwise, they won't have enough food for you. The residents of Floreana are quiet-spoken, generous, subtly good-humored and deeply principled. Several years ago, when an entrepreneur from another island stiffed local workers out of their pay, no one on the island would serve him food, no one would rent him a room to sleep in and no one would speak to him. The entrepreneur's project collapsed. The island's quirky politics and fierce independence make such an endeavor socially daunting. As Campbell says, "It gets complicated real fast."

A recurring mantra in the recent National Academies report on gene drive—and, indeed, in almost every official white paper about genetic engineering in the past four decades—is the need for "public engagement." But that bloodless phrase does not begin to capture the passion and complexity of real projects in real circumstances. If eradications in general are hard, eradications on inhabited islands are *really* hard. That became clear to Campbell several years ago, during a small meeting with members of the Floreana community to discuss the *Proyecto*. One resident, adamantly opposed to the idea of having to remove livestock from the island, looked straight at Campbell and said, in unprintable language, "If you do this, I'm going to kill you." Campbell recalls the moment as "very conflictive."

The intensity of emotion does not seem entirely inappropri-

TUI DE ROY

© 2019 Scientific American

ate, given the magnitude of the disruption. Since 2012 authorities in the Galápagos, with Island Conservation, have been formulating what they consider to be the most complex eradication plan of an inhabited island to date. It's not just the contentious adults on Floreana who make it complicated. It's children, pets and livestock, in addition to endangered birds and lava lizards.

Consider the staggering environmental risks of a "traditional," nongenetic eradication. To eliminate every rat and mouse from Floreana, the project calls for helicopters to drop some 360 million one-gram (0.035-ounce) pellets of brodifacoum—in Campbell's words, "Basically, systematically paint the whole island"—multiple times (drones are now being considered, after a pilot project on nearby North Seymour Island). To minimize potential health and environmental risks, the plan calls for extreme precautions. Water resources must be protected. Children may have to be removed from the island for up to six weeks. Pets will either need to be removed or restricted to domiciles or cages. Large agricultural livestock, such as cattle, pigs and horses, will have to be restricted in corrals (after the farmers of Floreana made clear that sending animals off the island for six months was not an acceptable option). Chickens will be housed in new covered coops, some of which have already been finished. Giant Galápagos tortoises in the Asilo de la Paz refuge will have to be temporarily restricted. Endangered birds will be trapped and held in specially built aviaries during the aerial baiting. In places off-limits to aerial baiting, such as buildings, homes or other structures, the eradication team will deploy traps and bait stations (the location of each bait station, in each home, has to be specified, and Carolina Torres, the lawyer for Island Conservation, is collecting signatures on draft agreements with each and every household). "A single pregnant female, or a single area missed, is a failure," Campbell says. "You need to get into every building, in every house, in every crawl space, in every closet, under every fridge to get every mouse."

The people from Island Conservation have taken the idea of "public engagement" to a new level. On a recent trip, Torres brought chocolates for Ericka Wittmer, a matriarch of one of the island's oldest families, and paid house calls to several island farmers to explain a legal issue involving contracts with the tenants who worked on parcels of their land. The organization recently provided paint for local homeowners to beautify their cinder block houses. When one resident expressed interest in starting a restaurant, Campbell and Torres encouraged her and promised to be customers. The organization has enlisted architects to design new chicken coops for the island's farmers; each unit will cost about $22,000. Campbell has learned the hard way that one-on-one relationship building is the best way to involve people in the decision-making process on such a delicate project. "If you do a town hall type of thing, they'll absolutely butcher you," he says. "Two or three people dominate the conversation, you don't know what other people think, and then afterwards, you have to spend a lot time dealing with the misinformation."

Despite initial reservations, Campbell says, most residents on Floreana support the eradication plan. In the highlands, Holger Vera, the farmer, stands amid a grove of orange trees, pineapple plants and other crops, lamenting the rapaciousness of the local rodents. They eat fledgling corn plants, he says; they devour pineapples; they eat the tubers of yucca. "Now they are even eating the sugarcane," he complains. "They are eating everything. But if we get rid of them, we can grow everything." Vera was initially skeptical about the *Proyecto,* Torres says, but he now sounds enthusiastic. Even if he has to board his seven dogs? "Yes, yes," he replies. Similarly, Cruz—who owns 80 cows, 130 pigs, more than 200 chickens, 10 horses and two dogs—agrees with the plan and the way it has been discussed with residents of Floreana. "We feel we are on the same page in terms of what's going on," he says.

"Essentially we have verbal agreements" from nearly all the residents, Campbell says. The plan still awaits final approval from Galápagos authorities. He believes the project could have been launched earlier if funding had been secured in a timely fashion. (Costs are expected to be $20 million overall, but funding hiccups have now delayed it until at least 2020; Campbell estimates that each year of delay costs $1 million.) Despite funding uncertainty, the reality of the *Proyecto* sunk in when seven orange, 20-foot shipping containers arrived in Floreana in 2017. They are intended to store uncontaminated livestock feed, or silage, for use during the rodent eradication; some farmers have already begun to store animal feed in the containers.

Pulling off a project this complicated is like managing a bureaucratic ecosystem—balancing the regulatory piece, the public engagement piece, the logistical piece, the funding piece, the poison mitigation piece. That's why Campbell thinks the Floreana project is "maxing out" the capability of traditional eradication tools. And that is why, not infrequently, he will say, "If we engaged the gene-drive technology, the conversations would be simpler, and the answers would be much more pragmatic."

IV. THE DEVIL WE DON'T KNOW

CAMPBELL FIRST became intrigued by the possibilities of gene drive in 2011, when he sat in on a conference call between biologists at North Carolina State University and officials of the U.S. Fish and Wildlife Service to discuss a possible genetic approach to control a runaway mouse problem on Southeast Farallon Island, about 20 miles west of the California coast, near San Francisco. John Godwin, a North Carolina State neurobiologist who studies animal behavior, had learned of the Farallon issue while skimming the Internet in 2011. He happened to be at a university with an established infrastructure dedicated to experimenting with—and considering the ethical implications of—genetic manipulation. Two of his colleagues, Fred Gould and David Threadgill, were already discussing the possibility of tinkering with the mouse genome in an attempt to create mice incapable of producing female offspring. Two other colleagues, Jennifer Kuzma and Jason Delborne, became deeply involved in how to engage the larger world of stakeholders—government regulatory agencies, animal management officials, bioethicists and, of course, the general public—in considering the prospect of releasing genetically altered animals into the wild. Kuzma and Gould serve as co-directors of the Genetic Engineering and Society Center at North Carolina State.

To make a long story short, Island Conservation joined forces in 2016 with other international groups to launch the GBIRd—Genetic Biocontrol of Invasive Rodents—program. GBIRd scientists are "cautiously investigating" genetic tools to preserve island ecosystems. The advent of the gene-editing tool CRISPR boosted efforts to develop an alternative approach to eradication. Those efforts gained traction in July 2017, when the Defense Advanced Research Projects Agency gave the North

© 2019 Scientific American

FLOREANA LAVA LIZARDS are easy prey for the feral cats that stalk the island.

TUI DE ROY

Carolina State group $3.2 million to pursue gene drives for mouse eradication on islands.

The basic idea of gene drive seems counterintuitive to anyone raised on the notion of Gregor Mendel's pea plants and the random inheritance of genes from parents. You usually have a 50–50 chance of inheriting a gene from one parent or the other. In rare instances, however, certain genes are favored, or "selfish"—they are inherited at much higher rates than random sorting would suggest. One such gene (technically, a region of the genome) exists in mice on chromosome 17; it is called the T-complex, and it is inherited at a rate of 95 percent. It might theoretically serve as a smuggler's bible, allowing a second gene to be quickly introduced in a population.

In an eradication scenario, researchers could theoretically attach a second piggybacking gene to the T-complex and essentially drive that second trait into the majority of offspring. One such mouse gene, known as *SRY,* determines male gender, so stitching it to a selfish gene would create more and more males (and fewer and fewer females) in each generation, until a mouse population would be daughterless. One of the basic requirements of gene drive is that the time between generations in the target animal is short; mice certainly qualify because their time between birth and reproductive maturity is 10 weeks. If the mice in the lab can be manipulated to pass along a desired gene, such as one to produce a single gender, and if those mice are reproductively successful in the wild, that gene could be rapidly driven into a population.

That's a lot of "ifs," but Threadgill, now at Texas A&M University, has been pursuing precisely that strategy in mice. This so-called daughterless breed could eliminate a native mouse population without environmental poison, without offshore animal relocations, without all the logistical nightmares entailed by the Floreana project. Paul Thomas, a biologist at the University of Adelaide in Australia, has been exploring the use of CRISPR to inactivate genes related to female fertility in mice, an approach that could be adopted to produce a population of entirely infertile females. In addition, Godwin, the neurobiologist, is testing whether an engineered mouse will pass sexual muster with wild mice (he is currently working with a batch transplanted from Southeast Farallon).

Species eradication is by no means the only application of gene drive. Target Malaria is an attempt to engineer mosquitoes so that they are incapable of transmitting malaria; the group, with funding from the Bill & Melinda Gates Foundation, has already begun community outreach efforts in Africa in anticipation of a field test. But Massachusetts Institute of Technology biologist Kevin M. Esvelt, who proposed a gene-drive experiment in mice on Nantucket Island, now feels a field trial is too risky. In the gene-drive game, the rule of thumb is that islands are the best place for a field test; smaller islands are better than larger ones, and uninhabited islands are better than inhabited ones. Campbell suspects the first field test of gene drive will involve mosquitoes and adds that the U.S., Australia or New Zealand would probably be the most appropriate venue because their regulatory infrastructures are sophisticated enough to assess new hot-button genetic technologies.

Eradications are controversial, genetic modification even more so. "There is no safe way to experiment with these technologies in the wild," says Dana Perls, senior food and technology campaigner at Friends of the Earth. Jane Goodall, Fritjof Capra and other conservationists called for a moratorium on the research in an open letter published in September 2016. Firing a shot across the bow of Island Conservation, the signatories said they were "alarmed that some conservation organizations have accepted funding for and are promoting the release of engineered gene-drive organisms into the wild."

The great fear is "unintended consequences"—that something unexpected and bad will happen. There is no question that gene drive, as the National Academies put it, "may have harmful effects for other species or ecosystems," and that alone warrants cautious and prudent development. But in previous public debates over genetic technologies, such as the battle over recombinant DNA in the 1970s, it was often difficult to separate legitimate concerns from exaggerated fears.

© 2019 Scientific American

Back in the real world, during an excursion into the highlands of Floreana, Campbell and Torres led me to a freshwater spring—not far from the cave where the island's first settler, the drunkard Watkins, allegedly slept off his hangovers. As part of the project, the entire area surrounding the spring, which is already fenced off, will be covered with a tent, and special filters will be placed on the pipes to make sure no rodent bait gets into the system—even though brodifacoum is not water-soluble. Part of public engagement, Campbell said, is dealing with perceptions as well as legitimate fears. "You're working with people's perception of toxicants," he explained. "It's challenging to change people's perceptions of this, because they don't." One more reason, Campbell continued, that the genetic approach was more appealing. Then suddenly he changed the subject.

"Here we are," he said quickly, pointing to a rustle of vegetation inside the chain-link fence. "You see it? A rat!"

A pair of shiny, dark eyes briefly appeared amid the leaves. Campbell identified it as *Rattus rattus*—the black rat, which is known to eat the eggs and hatchlings of Galápagos petrels and giant tortoises. Like rats everywhere, it disappeared quickly—a sentinel of an inevitably larger population and a larger covert threat to what Campbell calls "species on the brink."

V. "THE STRANGER'S CRAFT OR POWER"

EVERY STROLL IN THE GALÁPAGOS is a nature walk, and each living creature tells a conservation story—some with happy endings, some not. During our last day on Floreana, a number of these stories began when Campbell's keen eye alighted on the animals that make this landscape so beloved—and beleaguered.

During breakfast, a cactus finch stalked our table. Its strong black and yellow beak had evolved to be larger and stronger, Campbell explained, to crack the unusually large and hard seeds of the local *Opuntia* cactus on Floreana; the cactus, in turn, is evolving even larger and tougher seeds to thwart this poaching—a reminder that evolution is not a textbook concept but an ongoing process. Moments later Campbell spotted a mouse darting behind a hunk of lava. As we finished our meal, another invasive species made an appearance—the sleek, black, smooth-billed ani (pronounced "Annie"). An example of old-school unintended consequences, farmers introduced the bird to the Galápagos in the 1960s in the belief that it could control ticks that afflicted cattle; it did not live up to its billing, so to speak, but it has exploded in numbers as an invasive species.

Later, on a walk to La Lobería Beach, Campbell pointed out fresh tracks of feral cats in the sand; they devour juvenile marine iguanas and lava lizards. ("The small ones have zero chance of getting away," he said.) Near the head of the beach, he indicated the gnawed-off limb of one of the *Opuntia* cacti. When rodents chew down the cacti, he explained, the plants fail to flower or bear fruit—eliminating a crucial source of sustenance for tortoises and mockingbirds, especially in the dry season, and depriving finches of nesting sites. And we paused to admire several magnificent sea turtles temporarily trapped in a lagoon during low tide. Their eggs and hatchlings, too, provide tasty meals for rats and cats.

It was Darwin's 20th-century bulldog, Richard Dawkins, who revived poet Alfred, Lord Tennyson's phrase "Nature, red in tooth and claw" to describe the noir side of natural selection—nature's game is not always pretty, and the postcard-perfect ecology of a place like the Galápagos often conceals a darker, more unsentimental interaction of predator and prey—an interaction whose delicate balance humans have repeatedly perturbed, whether by introducing invasive species or by attempting to atone for those ill-conceived introductions with literally toxic remedies. And now, on the horizon, we may have to decide whether to use futuristic techniques of genetic modification to restore the islands to an earlier, more pristine state.

For what it is worth, a small sampling of opinion on Floreana did not betray much local concern about the potential applications of gene drive, although it is not clear how well understood these technologies (and their potential risks) are. Vera shrugged off any worries and said he would have no problem with a genetic solution to the rodent problem. Ingrid Wittmer, another descendant of one of the earliest families on Floreana, shook her head no when asked, instead expressing concern about the fate of the short-eared owl once its main food source, mice, was eliminated during the *Proyecto*. Cruz, whose father emigrated to the island in 1939, when the population numbered 11, offered a farmer's perspective to the idea of daughterless mice: "It's like artificial insemination in cattle," he said. "If you want females, you use the semen for females. It's the same thing."

"For me, these are issues we've created, and to sit back and do nothing, there's going to be grave consequences," Campbell said. "We know where things are heading. To actually *not* do something is ... is just irresponsible. If you have the tool, and you don't use it, *you're* culpable."

We don't have the tool yet. But if the craft of molecular biology eventually captures the power of gene drive, and it is used to manage invasive species in the Galápagos or any island, it is worth remembering that almost every ecological catastrophe visited on the planet's living laboratory of evolution has come at the hands of humans. The goats, the donkeys, the rats, the cats, the pigs, the mules, the mice and, yes, even those short-lived ocelots arrived with human help, on human boats, through human agency.

In a wry observation that resonates nearly two centuries later, Darwin remarked in his journal that while birds in England had developed a well-earned distrust of humans, the birds in the Galápagos "have not learned [such] a salutary dread." He went on to offer what might serve as cautionary words about 21st-century science and gene drives in particular. "We may infer from these facts," Darwin wrote, referring to the lack of fear in birds, "what havoc the introduction of any new beast of prey must cause in a country, before the instincts of the indigenous inhabitants have become adapted to the stranger's craft or power." SA

Stephen S. Hall is an award-winning science writer and regular contributor. He is author, most recently, of *Wisdom: From Philosophy to Neuroscience* (Knopf, 2010).

MORE TO EXPLORE

Current Status of Alien Vertebrates in the Galápagos Islands: Invasion History, Distribution, and Potential Impacts. R. Brand Phillips et al. in *Biological Invasions*, Vol. 14, No. 2, pages 461–480; February 2012.

Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies of Sciences, Engineering, and Medicine. National Academies Press, 2016. www.nap.edu/23405

Current CRISPR Gene Drive Systems Are Likely to Be Highly Invasive in Wild Populations. C. Noble et al. in *eLife*, Vol. 7, Article e33423; June 19, 2018.

scientificamerican.com/magazine/sa

© 2019 Scientific American

CAN WE EAT INVASIVE SPECIES INTO SUBMISSION?

The tale of a giant Amazon fish reveals the promise and peril of "invasivorism"

By Michael Snyder

© 2019 Scientific American

PAICHE, which can weigh up to 400 pounds, is eating its way through freshwater fish populations in Bolivia. Now people are eating the paiche in an attempt to slow its rampage.

© 2019 Scientific American

LAS PEÑITAS, BOLIVIA—BEFORE HE HAD ever seen a paiche, fish trader Eric Salazar had heard the giant Amazon fish could grow up to 10 feet long, weigh 400 pounds and eat a man whole. The paiche, or *Arapaima gigas,* is the world's largest scaled freshwater fish. Native to the jungles of Peru and Brazil, it first appeared in nets in Bolivia's Amazon Basin in the early 1990s. As it migrated upriver, rumors traveled with it. People said it was created by nefarious Peruvian scientists, that they fed it with the blood of farm animals, that it wasn't a fish at all but a monster.

They weren't entirely wrong. The paiche is carnivorous—although it eats other fishes, not humans. And it did enter Bolivia from Peru, where it had been added to the Convention on International Trade in Endangered Species index in 1975 as a species prone to extinction if trade was not closely controlled. A few years later a flood washed juveniles out of a Peruvian fish farm near the border into Bolivia's watershed. By the time Bolivian fishermen noticed the strange creature, it was already established in the oxbow lakes and seasonal lagoons that dot the forest.

Since then, the paiche has spread across more than 45,000 square miles, roughly a quarter of the Bolivian Amazon. There are no official data yet on its impact, but there's plenty of anecdotal evidence of environmental damage. Fishermen who have worked these waters for years say the native species they prefer to eat—particularly giant Amazon catfish such as surubí, bagre and pintado—have become scarce. Others, they say, have disappeared entirely.

In the past decade paiche meat has also become more popular among city dwellers. Conservation-minded chefs in the cities promote paiche as a sustainable choice, which is true at face value: The fish is a nuisance, and other edible river fishes are disappearing. The idea is to control or maybe even eradicate the fish by deliberately overfishing it, but Salazar, who profits handsomely from the paiche's lean, white meat, says that would be impossible. "To eradicate the paiche," he says, "would be to pull a star from the sky."

Bolivia is now faced with a controversy that, for the past decade, has been a central debate in the young field of invasion ecology here in U.S.: Could human hunger help control the spread of invasive species, or will the rules of capitalism, combined with our unquenchable thirst for more, only make matters worse?

IN BRIEF

Paiche, a carnivorous fish native to Peru and Brazil, has spread throughout more than a quarter of the Bolivian Amazon; simultaneously, local fish populations are disappearing. **"Invasivorism,"** or eating invasive species as a means to control or eliminate their numbers, has become a popular tool, utilized by restaurants and even Whole Foods. **Research on the efficacy** of this approach is limited, and the complexity of ecosystems requires careful management of invasive populations.

PRECEDING PAGES: GETTY IMAGES

EAT THEM TO DEATH

AN INVASIVE IS ANY SPECIES introduced by human intervention that has caused economic or ecological damage by growing superabundant in a nonnative habitat. Invasives can be fish, bivalves, mammals or plants. They can be as sinister as kudzu ("the plant that ate the South") or innocuous as dandelions. They can be as delicious as wild boar; as unappetizing as the parasitic sea lamprey sucking blood from

© 2019 Scientific American

NADIA DEL POZO AND FELIPE LUNA

native fishes in the Great Lakes (they're a delicacy in England); or entirely inedible, like the tiny zebra mussels clogging pipes and choking native shellfish throughout the upper Midwest.

Invasive species have followed us around the globe for as long as we have been mobile. They've hitched on the hulls of transoceanic ships, and we've carried them home with us deliberately, introducing them for food, farming and recreation. Invaders are now the second-most important cause of global biodiversity loss after habitat destruction, and the more we move about, the more they spread. Conservative estimates have invasives costing the U.S. tens of billions of dollars annually.

Among the first scientists to promote gastronomy as a tool to combat invasion was Joe Roman, a conservation ecologist at the University of Vermont. His 2004 article for *Audubon,* entitled "Eat the Invad-

FISHERMAN just outside of Las Peñitas cleans a piranha, one of the few fish local people like to eat that has not been decimated by the paiche.

© 2019 Scientific American

ers," articulated a simple argument: If we can hunt native species to extinction, as we have for eons, why not deploy our insatiable appetites against invaders?

Roman's modest proposal had little impact when it first appeared. Yet as interest in food ethics, locavorism and foraging grew, the elegant logic of "invasivorism" hit a cultural sweet spot. In 2005 Chef Bun Lai created an invasive species menu for his sushi restaurant, Miya's, in New Haven, Conn. In 2010 the National Oceanic and Atmospheric Administration launched its "Eat Lionfish" campaign to combat the species' invasion of the Caribbean. In 2011 Food & Water Watch hosted an invasive species banquet at the James Beard House in New York City. In 2012 Illinois extracted 22,000 metric tons of invasive Asian carp and sold it to China, where it is commonly eaten, for $20 million.

Other projects have taken a more participatory approach: The University of Oregon's Institute for Applied Ecology hosts an annual Invasive Species Cook-Off (aka Eradication by Mastication); Web sites such as invasivore.org—run by Matthew Barnes, a biologist at Texas Tech University—and Roman's own site, EatTheInvaders.org, promote home recipes for exotic species. Even Whole Foods has gotten onboard; in 2016 the upscale grocer added lionfish to the shelves and started promoting it as "an invasive species" in the Atlantic Ocean and Caribbean Sea, "far from its native waters."

ACTIVISM OUTRUNS DATA

BUT POPULAR INVASIVORISM has also preceded solid scientific study. "I don't think we have the data yet to know if this is successful," Barnes says. "There are a lot of small experiments going on but no large-scale data gathering." What data do exist are less than conclusive. In 2013 a group of researchers from the Netherlands working on the southern Caribbean islands of Bonaire and Curaçao found lionfish biomass near Bonaire in areas where harvest was encouraged was a third of that in areas where it wasn't and less than a quarter of what it was in the waters surrounding Curaçao, where the local government has yet to begin control efforts by harvesting. Those numbers were promising. Yet in 2016 Fisheries and Oceans Canada wrote in a report there was an "extreme" risk of Asian carp species establishing populations in three of the five great lakes within 50 years, despite millions of dollars spent by the U.S. government to build aquatic barriers and promote harvest programs. Marketing aside, most people still find the fish unappetizing.

Invasivorism can also overlook some of the complexities of ecological invasion. Different types of invasives present a variety of problems. Picking the leaves or fruits from plants such as dandelions and Himalayan blackberries does nothing to keep them from growing. Telling people they should eat Asian carp won't make the animal appealing. And even if you could convince people to eat nutria—the South American swamp rat introduced in the 1940s to clear Louisiana's Mississippi Delta waterways of another invasive species, water hyacinth—there's no way they'd be able to catch up with the population, which has already wiped out entire swaths of native greenery.

There is evidence that harvesting programs could be effective if started when the target population is still small and concentrated. In their cautiously optimistic paper for the U.S. Fish and Wildlife Service, biologists Susan Pasko and Jason Goldberg noted that between the years of 1981 and 1989 the U.K. successfully eradicated a nutria infestation by incentivizing trappers, although they don't mention whether or not anyone ate the animals. Yet a similar program targeting invasive minks failed just a few years later because that animal had already dispersed too widely. "Incentive programs can only be successful," the authors wrote, "if the number of individuals harvested exceeds the number that would normally not survive during a single breeding cycle." For Caribbean lionfish, that might require removing up to 65 percent of the species. With plants, those numbers are even higher.

BACKFIRE: EVIL FISHES BECOME POPULAR FARE

THE WORST-CASE SCENARIO of invasivorism is not that it won't work, rather it will make a troublesome species popular. Martin Nuñez, an ecologist at the University of Tennessee, has published several papers warning of perverse incentives to distribute economically valuable species more widely. "If you make money off a species," Nuñez says, "then that's an incentive to help it spread." Consumption is one of the most powerful incentives of all. Even advocates of invasivorism like Roman and Barnes raise the same concern.

In Hawaii, for example, feral pigs run rampant. Descendants of Eurasian boars from Russia and introduced for sport hunting long ago, they cause substantial damage to indigenous habitats. Yet to eradicate them would also be to kill an important part of the island's cultural lexicon, built around the practice of hunting and roasting the animals. Pasko and Goldberg point out it could also create new environmental problems. Feral pigs eat the flammable, invasive grasses that now cover large swaths of the islands; without them, fires could become more common and destroy more native habitat than the pigs do themselves. In the continental U.S., people continue to spread wild boars by introducing them as game. They now cause an estimated $1.5 billion of damage annually and, within 50 years, will have reached every county in the country.

The same has begun to happen with Bolivia's paiche and on a much shorter timescale. Paul Van Damme, a biologist working on a fisheries management program called Peces Para la Vida (Fish for Life) with the Bolivian organization Faunagua, has already seen a second wave of paiche invasion. "We thought it would take 50 or 60 years for the paiche to

© 2019 Scientific American

arrive in the upper Mamoré or Iténez," he says, referring to Bolivia's other two lowland river systems. "But the species is already there." This second invasion had multiple origins, among them poorly managed fish farms in Brazil where the species is also native and also overfished. Along Bolivia's northeastern border, formed by the Mamoré, fish farmers from Brazil cross the river to purchase baby paiche to stock their farms; on a 2018 trip to the region, some fishermen told me they had thrown those juveniles overboard when they saw authorities approaching, to avoid fines for transporting a protected species. In a particular case, he says, "this one guy actually distributed the species to farmers all over the basin," with promises of boom times for paiche meat ahead.

He wasn't wrong; paiche does have commercial potential. In the U.S., Whole Foods has been pushing farmed Peruvian paiche; a 2016 blog touts its "great back story" as a threatened species "rescue[d]" by sustainable farming. The grocer planned to double its stock for 2017. But already by 2014 a widely publicized study out of Peru and Brazil had found the paiche was extinct in 19 percent of the 80-plus communities surveyed and "depleted" in 76 percent. A 2010 study by the United Nations's Food and Agriculture Organization suggested that farming would have positive effects on wild paiche populations by redirecting harvest pressures to farmed populations. But fish farms are also notoriously difficult to regulate and prone to accidental release: Asian carp in the Midwest (introduced to clean algae from the farms), Louisiana crayfish in China and Atlantic salmon in the Pacific Northwest have all done substantial environmental damage after escaping from fish farms. The same fish farms that "rescued" the paiche in Peru might also ultimately destroy Bolivia's Amazon.

In the areas of Bolivia where the paiche first appeared about 25 years ago, it now accounts for 90 percent of wild catch, although it is unclear whether that's because other fish species are gone or because local fishermen are successful in focusing on the invader. In places where the fish arrived more recently, its growing market value has drawn more fishermen to the river, increasing pressure on paiche, along with native species. In other places, Van Damme says, paiche has become an alternative to blanquillo, a native species typically fished using freshwater dolphin meat as bait. In still others, paiche has driven both local fishes and local communities away from the water entirely. Even a single invasive species in a single river system can have staggeringly diverse effects.

The goals of conservationists and activists are no less varied. Roxana Salas, who heads Faunagua's legal arm, helped to lobby for the passage of Bolivia's first ever Sustainable Fisheries and Aquaculture Law, which passed in May 2017. That law was an important first step, laying the groundwork for better enforcement of embargoes on native species and encouraging internal trade in paiche, yet it included little in the way of concrete regulation. In December 2017 the Bolivian legislature passed a resolution that would allow communities within the boundaries of Manuripi National Park in the northern Bolivian Amazon to sell paiche for profit throughout the year, in an attempt to generate income and to put pressure on the invader. Salas's team is working to craft laws that promote economic growth via sustainable paiche fishing.

Fish and Wildlife's Goldberg describes "adapting to irreversible ecosystem changes" as "a means of last resort." For Chef Bun Lai, invasivorism is a way of "shifting our appetites" away from the unsustainable species we currently favor. Barnes and Roman see it principally as an awareness campaign—"a way to get [invasives] on people's radars," as Roman says. They all agree that preventing invasives from arriving in the first place is the only real solution and that invasivorism will only be an effective management tool in cases where the culprit is caught early. "Invasivorism is not going to save the planet. and it's not going to solve all of our problems," Roman says. "It's a tool in the toolbox. But, boy, you gotta be careful."

Tennessee's Nuñez, for his part, is skeptical of the founding premise. "The idea is that if we can drive a native species to extinction by eating it, we could do the same with an invader—but it's not obvious to me that those dynamics would be the same," he says. "Something can be intuitive and also be wrong."

In Las Peñitas the intuitive choice is to fish the new species away and restore the environment to its previous balance. But it doesn't take much work to reveal the flaws in that solution. In the first place, no environment is static, and the notion of balance is, as Barnes notes, "a social construct, scientifically tough to defend." Many local fishermen see paiche as a boon, a lucrative commodity in a region otherwise starved for resources. It is obviously preferable to preserve native species but also imprudent to ignore the economic potential a species might bring to a poor region of South America. There are plenty of incentives for fishing paiche. The question is whether they will do more harm than good.

Managing invasive species is, in the end, principally a question of managing humans. As Salazar and the many other fishermen of Las Peñitas asked out loud, time and again, "Who knows which is the worse predator—the paiche or us?" SA

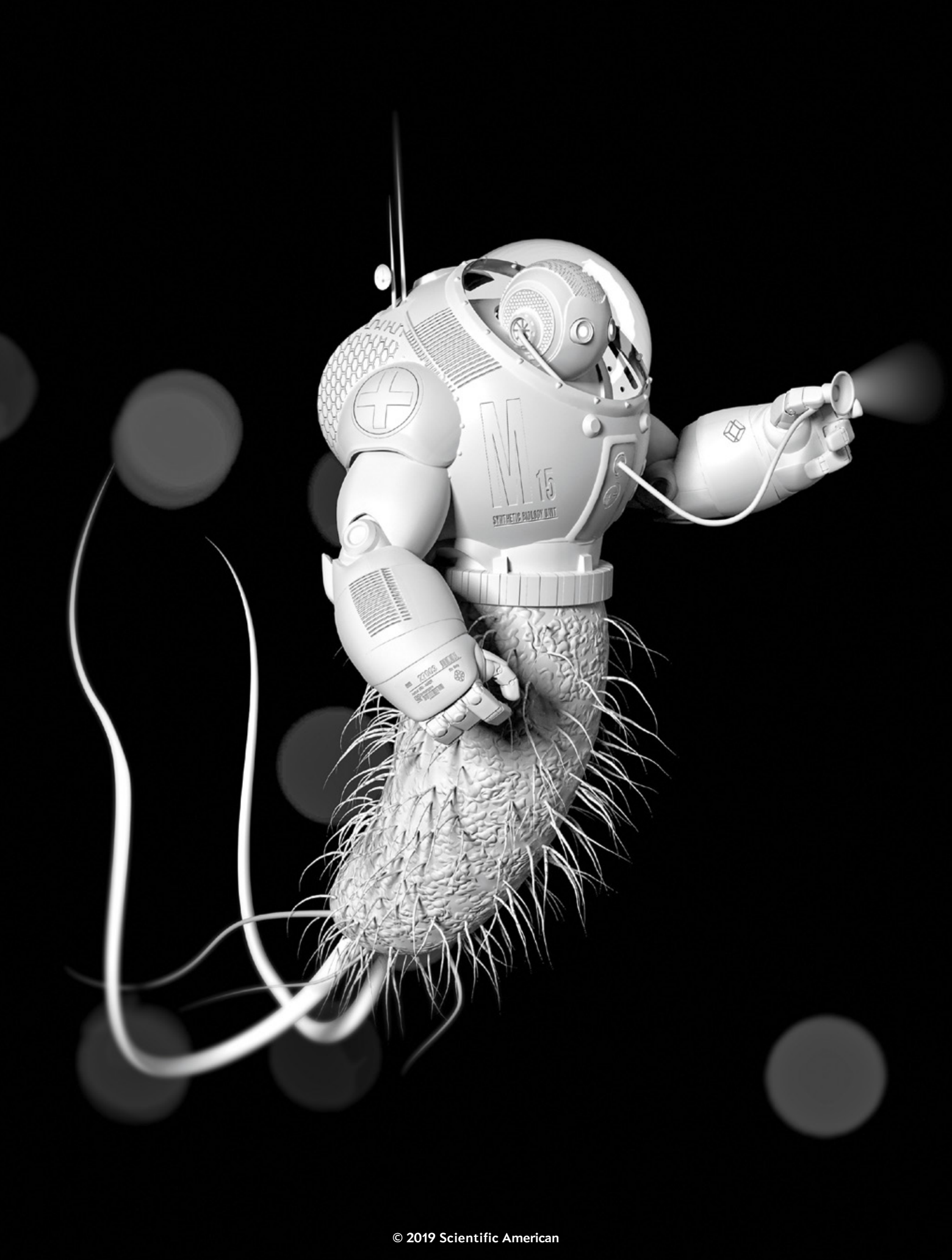
Michael Snyder is a freelance journalist in Mexico City. His work has appeared in the *New York Times*, the *Washington Post*, *Lucky Peach*, *Roads & Kingdoms* and Slate, among other publications.

MORE TO EXPLORE

Understanding Fishing-Induced Extinctions in the Amazon. Leandro Castello et al. in *Aquatic Conservation: Marine and Freshwater Ecosystems*, Vol. 25, No. 5, pages 587–598; October 2015.
Invasive species portal at the U.S. Fish and Wildlife Service: www.fws.gov/invasives

scientificamerican.com/magazine/sa

© 2019 Scientific American



© 2019 Scientific American

TRANSFORMERS

By reprogramming DNA inside harmful microbes, biologists are turning them into patient-saving drugs

By Michael Waldholz

Illustrations by GUYCO

© 2019 Scientific American

IN RECENT MONTHS SEVERAL DOZEN test subjects have volunteered to gulp down billions of tiny, toxin-gobbling contraptions built to cure a crippling disease. The devices are not made from the usual machine parts of metal, wire or plastic. They are rebuilt organisms: bacteria, reconstructed from the inside out to perform an intricate feat of medical care.

Researchers working at Synlogic, a Cambridge, Mass., biotechnology start-up, have been handing patients daily doses of a drink loaded with billions of *Escherichia coli* bacteria. These kinds of microbes typically bustle about our innards, occasionally causing infections but generally living innocuous lives. What makes these particular *E. coli* different is that scientists have revamped chunks of their DNA—genetic instructions that tell the microbes what to do—to transform these cells into engines relentlessly driven to devour poisonous loads of ammonia in patients' bodies.

The bacteria-based treatment is for patients with urea cycle disorder (UCD), a liver enzyme deficiency that can kill newborns and make adults sick. They are born with a faulty gene that produces defective enzymes unable to break down the nitrogen in high-protein foods such as meat, eggs and cheese. Normal enzymes turn excess nitrogen into a chemical called urea, which gets peed away. But for those with the genetic disorder, the excess nitrogen does not leave the body. Instead it generates toxic levels of ammonia that accumulate in circulating blood and inflict havoc when they hit the brain.

Synlogic's engineered bacteria will guzzle extra ammonia. Gut bacteria take in small amounts of ammonia already, using its nitrogen for growth. The retrofit from scientists gives the microbes a new genetic "circuit," a series of genes and regulatory bits of DNA such as volume controls and on/off toggles wired together like transistors in an electronic gadget. Wedged into the ordinary *E. coli*'s genome, the circuit replaces the bacterium's normally slow ammonia-consuming mechanism with a supercharged version, an ammonia-gobbling beast that switches on when it senses the low oxygen levels characteristic of the human gut.

If Synlogic's genetically altered bugs can gorge on ammonia in humans as they have in studies with mice and in initial human tests, then tossing down the bacterial concoction every day for the rest of their lives may enable UCD sufferers to survive practically symptom-free. The amplified bacteria will cure a devastating genetic disease, arising in more than 100 new patients a year in the U.S., for which there is not now an adequate remedy. "We've replaced a missing physiological function with a whole new kind of therapy," says Paul Miller, Synlogic's former chief scientific officer. "It's an amazingly powerful way to attack disease." Synlogic is crafting similar circuits against more common illnesses such as irritable bowel disease, inflammatory and immune disorders, and even cancer.

Transformed bacteria have a key advantage over more typical drugs, which are chemical-based pills where the only thing doctors can change is the dose. The bacterial circuits can be easily fine-tuned to increase potency or to extend or reduce the time of activity, and they can be turned down so that they become safer. Bacteria's natural ability to sense and respond to their environment also makes them target-specific: they can be programmed to release a therapeutic substance only when at the site of disease. This selective action may avoid the side effects

IN BRIEF

By taking control of a microbe's genetic circuitry, biologists can turn it into a medical treatment that switches on and off in particular situations.

The change involves connecting protein-coding genes and switches, in the way electrical circuits link conductors, resistors and capacitors.

Bacteria are now being modified with new circuits that let them treat genetic diseases, attack tumors and detect antibiotics.

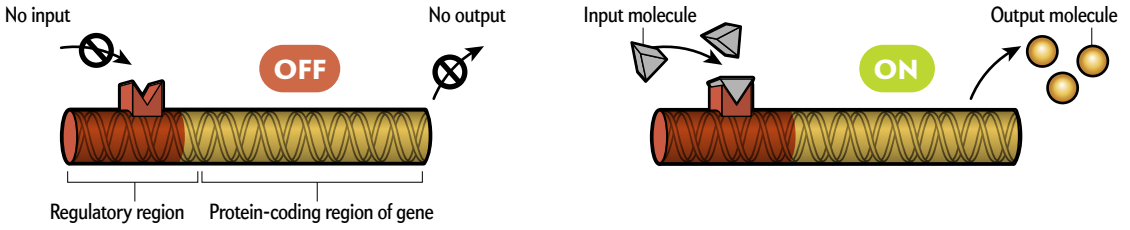
© 2019 Scientific American

Living Circuits

Genes—and other segments of DNA that switch genes on and off—can be wired together in novel ways. They work like electrical circuits that run household gadgets. These DNA circuits, though, can be placed within living organisms like bacteria to control the microbes' behavior. With that control, synthetic biologists can turn the organisms into living medicine.

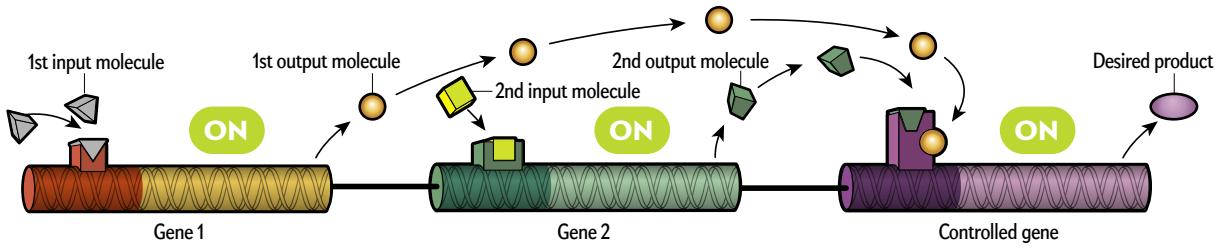
A Simple Switch

In a basic circuit, a gene can be turned on by a particular signal to produce a useful substance. The gene (*orange*) is linked to a regulatory region (*red*). When that region has no input, the circuit is off and produces nothing. But if that region is stimulated by an input molecule (*gray*), it turns the gene on and makes a desired output molecule.



Adding Complex Logic

The switch can be combined with other elements to give biologists more advanced control—logic—over what a microbe does. One example is this "AND" circuit. Gene 1 (*orange*), when switched on, sends output to the controlled gene (*purple*). Gene 2 (*green*), also switched on by an input molecule, sends output to the controlled gene as well. The controlled gene switches on only when stimulated by both gene 1 and gene 2.



Building Bacteria to Fight an Enzyme Disorder

Patients with urea cycle disorder have an enzyme deficiency that lets toxic levels of ammonia build up. Biologists are treating this by making *Escherichia coli* bacteria eat the ammonia. The microbes are engineered to produce large amounts of an amino acid, arginine, and they need to consume ammonia to make it. First a gene (*pink*) that inhibits extra arginine production is turned off. Another gene (*green*) is added, and it switches on when stimulated by a protein called FNR (*yellow*). FNR only does this in a low-oxygen environment such as the human intestines. When the entire synthetic circuit is placed in bacteria, they become arginine-producing machines only when stimulated by ammonia and low oxygen levels. The dual control ensures bacteria do this inside the body, not after they are excreted into the oxygen-rich outside world.

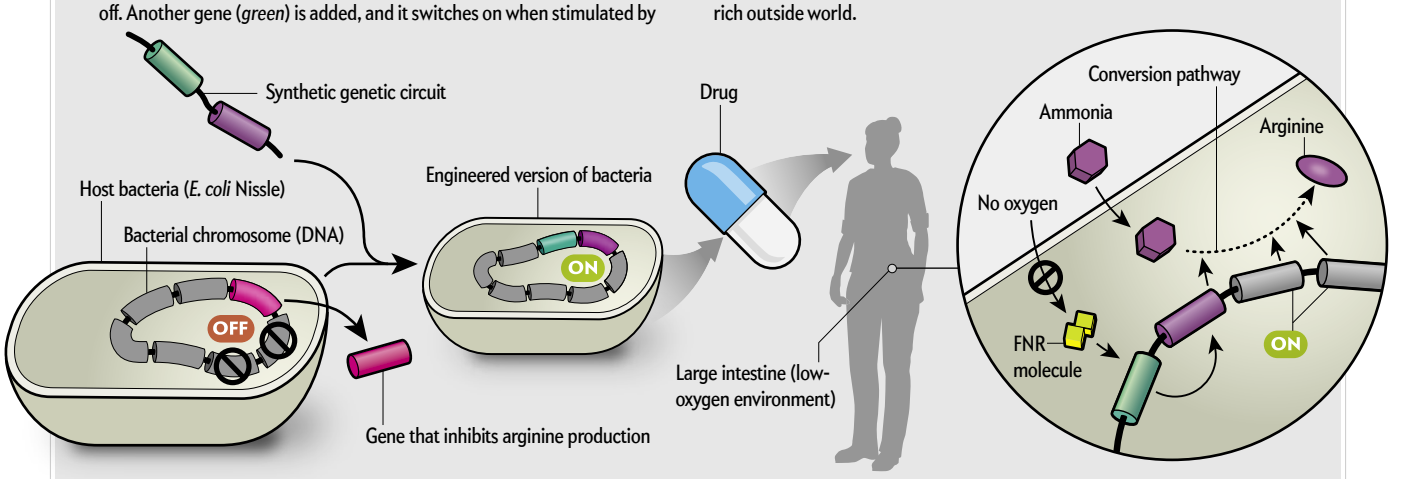


Illustration by Jen Christiansen

© 2019 Scientific American

typical of pills that act throughout the body.

The bacteria also may be able to replenish themselves within the human body, something no pill can do. They must still pass safety tests, and researchers acknowledge that they must show that their genetically enhanced bugs will not get unleashed dangerously into the environment. The Food and Drug Administration gave Synlogic the go-ahead to try the therapy in people in 2017 because the strain of *E. coli* being used in the UCD therapy has long been safely prescribed as an oral probiotic to treat inflammatory bowel disease. If the human tests pan out, the company's therapy-in-a-germ will represent the first clinical application to emerge from a relatively new branch of genetic engineering called synthetic biology.

The field rides on advances in manipulating DNA, giving scientists new laboratory tools to link stretches of DNA together and produce effects more powerful than simply changing one gene. "Synthetic biology is now producing some impressive accomplishments," says James Collins, a professor of medical engineering at the Massachusetts Institute of Technology and a leading researcher in the field. Human cells, for instance, have been fitted with enhanced DNA circuits to pump insulin into the bloodstream more precisely than daily injections for diabetics. *Salmonella*—the bacterium associated with food-poisoning outbreaks—has been rejiggered to sneak up on cancer cells and unload a cargo of toxic drugs. The DNA-circuit approach can also diagnose disease: researchers in Boston recently redesigned a microbe to alert doctors to early sepsis infections brewing in the blood of hospitalized patients. Existing tests rarely pick up the problem until patients are much sicker and hard to treat.

The new technology has the potential to be transformative not just for bacteria but for medicine itself. "Biomedicine sits on the cusp of a new revolution in medical care," says Wendell Lim, director of the Center for Systems and Synthetic Biology at the University of California, San Francisco. "Microbial and human cells are becoming versatile therapeutic engines." It was not always such a rosy picture, however.

Unnatural Responsibilities

Synthetic biology offers unusual rewards and risks

By Kevin M. Esvelt

The bold dream of synthetic biology is a world in which all living things can be reliably engineered in ways that help everyone and everything. In this dream, we can use genetics to program living organisms: "if condition A is met, then do action B." To give a near-term example, bacteria might produce a medicinal protein only in the presence of indicators of a particular disease.

Why use living systems and not a vat of chemicals? Because natural systems routinely perform complex chemistry that scientists can only envy, and they do it at room or body temperature, without the need for toxic chemicals or outside aid. Better still, living factories are far more energy-efficient than anything made of silicon and metal. Biology is fast, clean and green. And we should use such systems because people and ecosystems are alive, and the best way to repair life is with life. To fight an evolving pathogen, use an evolving cure.

There are problems, though, in bending nature to our own ends. Adopting an organism to work for us means it is using energy that could otherwise be spent replicating, so it will not reproduce as well as competitors. Evolution will constantly select for faster-reproducing mutants that no longer do what we want. Biology's greatest strength is its capacity to replicate and evolve, but that also presents the greatest challenge.

One way around this is to incorporate limits on the ability to change, particularly for those few cases where our changes might be able to spread in the wild. For example, one approach is to employ unnatural amino acid tethers: they make essential proteins within cells wholly dependent on chemicals that do not exist in nature. If the amino acids are withheld, the proteins will not function, and the bacteria cannot grow out of control.

We are also better at building within the scope of evolutionary limits: microbes are now programmed to release a burst of complex molecules and then die, mostly avoiding evolutionary selection against production. Cellular pathways can be reworked to eliminate most unwanted side effects. Engineered viruses that target bacteria will kill invading pathogens, multiply until the invaders are gone and then stop, leaving the patient untouched.

We must also be careful to make sure benefits always outweigh the risks of reworking organisms. Mistakes are inevitable. Thus, the projects have to be worth it, especially the earliest examples that must justify the technology to the world. Bacteria can be built to make a slightly cheaper flavor of vanilla, but is that a significant boon to humanity or the environment? This is likely not enough to be a pioneering example of a novel technology or to justify its use. On the other hand, building cells that can selectively destroy cancer or cure diabetes is something everyone can get behind.

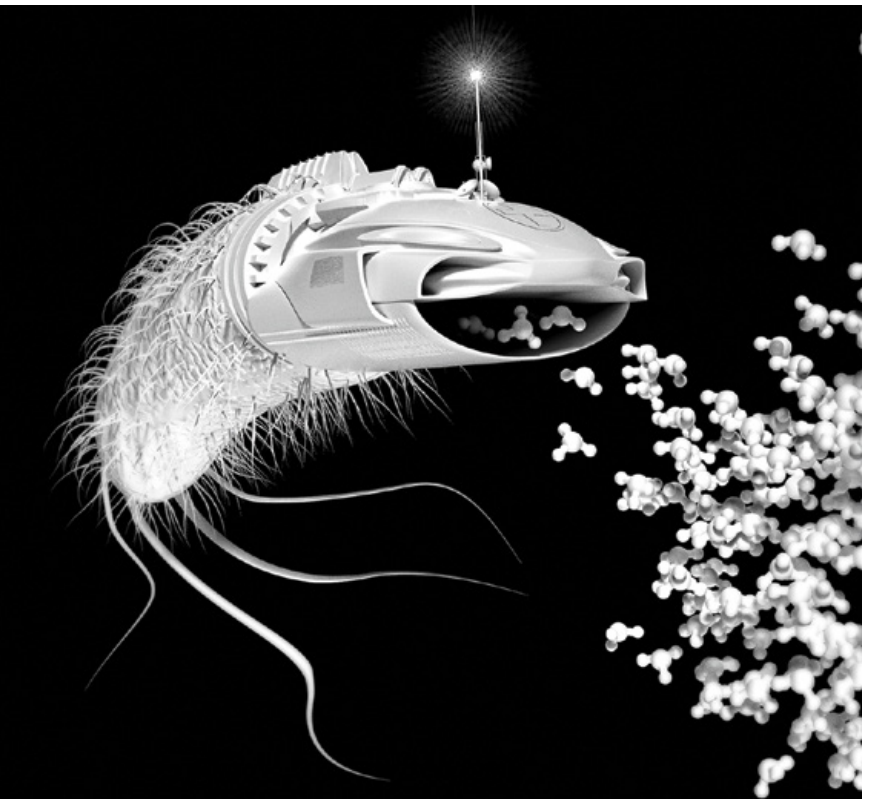
The greatest biological risk to civilization stems from pandemics of infectious disease. Until now, these were inevitable, but we might soon use biotechnology to stop them. Ordinarily, a person's body confronts an invading pandemic pathogen by evolving its own defenses, creating a whole series of antibodies in the hope that one will effectively neutralize the invader. It is a

ENGINEERING BIOLOGY

FOR THE PAST 40 YEARS SCIENTISTS have used genetic engineering to discover and manipulate genes to reveal the intricate machinery that rules all life. But they were limited in their understanding of how the different parts fit together and worked in real life. Things that looked good in a test tube fell apart when tried in a real cell or animal. When synthetic biology got started, there was a lot of early hype, Collins admits. But beginning about 20 years ago, he and like-minded biologists, fueled by advances in sequencing and synthesizing DNA, have been employing the newly discovered genes and other DNA elements as interchangeable components to design and build medical applications that actually work outside a glass dish.

Part of the change has come from scientists with a bent for

© 2019 Scientific American



process of trial and error that takes time; this is why you are typically sick for three to four days before getting well. Sometimes that is just too long, and people die. A better strategy is to give the human body a head start: Take the genes for several known protective antibodies, put them into the harmless shell of a virus and inject that virus into people. The virus enters their cells, which then start to churn out already optimized protective antibodies against the invader, ending the threat.

Finally, as scientists we need to respect the fact that engineering life unsettles many people. That means we must consider social risks as well as technical ones. We cannot just explain what we are doing—that only convinces other scientists. Instead we must relate why we care, who could benefit and what the risks may be. Above all else, we should actively invite concerns and criticism from the earliest stages because no matter how great our expertise, we cannot reliably anticipate every consequence on our own. At its best, science is a fundamentally shared undertaking. If we are to engineer life, let us all decide how to do it together.

*Biologist **Kevin M. Esvelt** leads the Sculpting Evolution research group at the Massachusetts Institute of Technology's Media Lab.*

tinkering like engineers. "There's been a convergence of new ideas in the past few years that's driving the field," says Jeff Hasty, who co-directs the BioCircuits Institute at the University of California, San Diego. Hasty started his science career over two decades ago with a Ph.D. in physics. Only partly joking, he describes himself now as a "hybrid computational/molecular biologist." Synthetic biology is populated with folks such as Hasty, who embrace an engineer's inclination to "make new stuff," he says.

"Much as an electrical engineer uses conductors, resistors and capacitors to create new electrical devices," Collins says, "we put together the components of biology—genes, proteins, RNA, transcription factors and other DNA—to create a particular function."

Collins notes that electronic gadgets are useful models for understanding genetic circuits. Consider an air conditioner thermostat. It senses an input—warming air temperature—and responds with an output—turning on the AC. When the air is cooled, the thermostat switches the machine off. Single-cell microorganisms such as bacteria survive in a similar way. Ever alert to an input, say, the presence of a competing germ, a bacterium responds with an output, secreting a natural antibiotic to kill its enemy.

The circuit builders of synthetic biology separated from straight genetic engineers as a result of coincidental insights by Collins and another research team. In 2000 Collins's lab, which was then at Boston University, reported making a genetic "toggle switch," one of two synthetic gene networks published in *Nature* in January of that year. The twin reports (the other was from a group at Princeton University) are generally cited as launching synthetic biology because they showed that "we could take parts of cells and link them together to generate a novel circuit the way an engineer might," Collins says. (It is no coincidence that, at the time, he was surrounded by circuits. He was running a bioengineering lab that was designing mechanical limbs for the disabled. Today Collins works at synthetic biology facilities in three different institutions in the Cambridge area. And he has trained about two dozen scientists—among them Hasty—who now have their own operations.)

In the years following the first primitive DNA-based switches, the still small community of synthetic biologists entered into a can-you-top-this competition, cooking up increasingly complex circuits that harnessed cells' natural sense-and-respond behavior. "As we went along, we learned, more than we first realized, how remarkably versatile the cell is," U.C.S.F.'s Lim says. He describes the cells as adaptable automotive "chassis" into which researchers can swap different genetic engines to carry out therapeutic functions.

One of the first commercial applications emerged in 2006 from scientists led by Jay Keasling of the University of California, Berkeley. Backed with a $42.6-million grant from the Bill & Melinda Gates Foundation, Keasling's lab refashioned the metabolic pathways of ordinary baker's yeast with lab-designed cir-

© 2019 Scientific American

cuitry that turned sugar molecules into a critical ingredient for making the malaria drug artemisinin. Previously, the precursor molecule for manufacturing the drug was extracted by hand from sweet wormwood plants native to Asia, a costly process that made the drug too expensive for use in poor regions where malaria is rampant. "It was a breakthrough," Collins says. "It was the first time a network of genetic material, not just one gene at a time, was used to transform a microbe—the yeast—into a solution for an important real-world problem."

BROKEN CIRCUITS

BUT IT DID NOT LAUNCH a revolution. At about that time, J. Craig Venter, a famous genome scientist and co-founder of Synthetic Genomics in La Jolla, Calif., joined the synthetic biology fray, giving the technology its first public star. His highly publicized objective, which garnered a whopping $300-million investment from Exxon in 2009, was to make gasoline from algae found in pond scum. In 2010 Keasling received a $134-million grant from the Department of Energy to fund research aimed at coaxing yeast cells to synthesize diesel from chemicals in sugar plants. Earlier that decade Keasling had co-founded Amyris, a biotech company in Emeryville, Calif., to commercialize the alternative fuel technology.

Both projects wound up giving synthetic biology a bad rap. After four years, Exxon and Venter, as well as Amyris, essentially gave up the synthetic oil project. The cost of scaling up commercial production, as compared with the current low price of oil and natural gas, has forced Amyris and several other biofuel-from-microbes start-ups to put the venture on hold. These companies were disasters for investors. Amyris and different synthetic biotech companies launched between 2005 and 2010 on the promise of making oil from bugs continue to produce notable advances in biocircuitry design. But their new genetic circuits are not as widely celebrated. Instead these one-time rock stars of synthetic biology are reconstructing microbes to fabricate chemicals used in manufacturing solvents and lubricants, as well as the principal ingredients for cosmetics, fragrances, detergents and over-the-counter health products.

While Wall Street investors and the science media largely focused on the headline-grabbing dreams—and the less dreamy waking reality—of biofuels from bugs, without fanfare Collins and his colleagues spent much of the new century's first decade working out technical hurdles for what was to come next: better medicine. After years of tedious experiments, in 2010 Collins engineered a bacterium that, in lab tests, weakened drug-resistant germs enough to make them vulnerable to existing antibiotics.

At about the same time, Tim Lu, another Collins postdoctoral protégé (with a Ph.D. in electrical engineering and computer science from M.I.T., as well as a medical degree from Harvard University), embedded circuits into a different kind of microbe, a virus that infects bacteria. Certain hard-to-treat infections arise when bacterial colonies encircle themselves within a gooey, protective biofilm. These bacteria may have evolved the biofilm to fend off the marauding viruses, which are called bacteriophages. Lu designed his circuit in the virus with a gene that codes for a biofilm-degrading enzyme. Lu's circuit also programmed the bacterial viruses to sense the presence of biofilm, infiltrate its defenses and respond by off-loading the film-busting enzyme.

Lu and Collins realized it might take years to perfect their infection fighters. But they also thought their bacteria might be ready sooner for another commercial use. In a meeting in 2013, Lu and Collins told a gathering of biotech funders at Atlas Venture in Cambridge that their labs' genetically enhanced microbes could be transformed into living sentinels able to provide early detection of disease within the human body or contaminants in the air or water.

The Atlas executives, however, were taken by another, related idea. They envisioned greater profits if the bacteria could be wired not simply to act as sentinels but to sense a health problem within the human gut—and then generate a therapy to treat it. The idea for Synlogic was born. In early 2015, about six months after the company hired its first researchers, it used Collins and Lu's inventions to create an early version of the UCD therapy.

THE CIRCUIT IS DESIGNED TO FIRST FABRICATE A CANCER DRUG INSIDE THE BACTERIUM. IT THEN DIRECTS THE MICROBE TO SLIP INTO THE INTERIOR OF A TUMOR, CARRIED BY THE BLOODSTREAM, AND SELF-DESTRUCT. WHEN THE MICROBE BURSTS APART, IT RELEASES ITS PAYLOAD OF DRUGS.

"I've been in the pharmaceutical industry a long time, and I've never seen a pharmacologic go from a scientist's idea to clinical testing in so short a time," says Bharatt Chowrira, president of PureTech Health and a former executive at Synlogic.

REPURPOSED PARTS

THE TREATMENT'S COMPONENT is an especially clever circuit assembled with genetic parts that biologists uncovered during decades of *E. coli* research. The Synlogic circuit changes the bacterium's usual ammonia-to-nitrogen-to-cell growth mechanism into a factory to churn out an amino acid called arginine. The researchers chose arginine because its cellular manufacturing demands more nitrogen than other amino acids. The need to make arginine turned the bacterium into an ammonia-gulping organism because it was desperate to take in nitrogen. With the circuit embedded into its genome, the microbe winds

© 2019 Scientific American

up producing 5,000 times more arginine than the normal strain of bacteria, Synlogic researchers say.

The circuit depends on a switch, a sequence of DNA that responds to a protein called FNR. Like the thermostat in an air conditioner, FNR is sensitive to changes in the bacteria's surroundings. It enables *E. coli* to respond to an environment that lacks oxygen. When FNR senses that the bacteria are in a low-oxygen environment such as the large intestine, it turns on genes the microbes need to thrive. When the bacteria move outside the body, where there is plenty of oxygen, FNR is silent. This is a safety mechanism designed to prevent runaway organisms with high growth rates. Once the microbes exit the intestines and hit our oxygen-rich atmosphere as feces, the entire system shuts down, and the *E. coli* die.

There was one problem, though, Synlogic's Miller says. *E. coli*'s genome contains a "repressor switch," a gene called *argR,* that shuts down arginine production when it senses the bacteria have enough. So designers needed a mechanism to deactivate *argR* in their new circuit. Synlogic researchers accomplished this by knocking out the long DNA sequence that surrounds and includes *argR* with a nearly identical stretch of DNA in which the *argR* gene is deleted.

Several synthetic biologists have come up with other genetic circuitry to deliver anticancer drugs deep inside tumors. U.C.S.D.'s Hasty has armed a strain of *Salmonella* bacteria that is not harmful to humans with a special set of genetic instructions. Hasty's experimental cancer therapy takes advantage of recent research that found that some bacteria often reside inside tumors. Scientists believe but are not certain that bacteria that naturally circulate in the bloodstream are attracted to tumors "because the environment provides a safe refuge from the immune system," Hasty says.

Hasty's genetic program forces the *Salmonella* to carry out a two-step process. The circuit is designed to first fabricate a cancer drug inside the bacterium. It then directs the microbe to slip into the interior of a tumor, carried there by blood the tumor needs for nourishment. At a moment directed by the circuit, the *Salmonella* self-destructs. When the microbe bursts apart, it releases a payload of drugs. "Sort of like a kamikaze mission," Hasty says.

In another ingenious bit of designing, Hasty added several genetic components to make the therapy self-renewing. "We introduced into the bacteria a 'quorum-sensing' system that can detect when *Salmonella* reproducing inside the tumor achieve a certain population," he says. When the multiplying microbes reach a high-enough density, the quorum sensor triggers the release of a protein that slices the *Salmonella* apart from the inside, spilling out the anticancer drug. This act of suicide kills most but not all of the *Salmonella*. Those that remain begin multiplying again, driving the cycle to repeat itself over and over.

The idea of attacking a tumor cell from within is especially attractive because most chemotherapy drugs work by eating away at a cancer cell's outer walls. In a study in mice, the bacterial therapy did not work any better than standard chemotherapy when delivered alone, Hasty says. "But when we combined it with chemotherapy, we observed decreases in tumor sizes and a 50 percent increase in life expectancy in mice with a type of metastatic cancer," he notes.

SEARCHING FOR APPROVAL

THE *SALMONELLA* WORK is still being refined. Synlogic's UCD treatment is much further along, and the FDA's approval process for this first therapy to involve genetically modified microbes is being scrutinized closely. The agency has released rules to regulate the microbe-based therapies under a new category it calls "live biotherapeutic products." Unlike other medicines (with the exception of some vaccines), the new therapies are composed of organisms that are alive and have the potential to mutate as they reproduce. Because of this, the FDA wants assurance that the makeup of the therapies will not vary from one batch to another. In addition, it wants proof that the microorganisms cannot survive in the environment by themselves, as Synlogic claims. "Many of us are watching how Synlogic is handled by the regulators," Hasty says. "If they can't get their therapy approved, we may all be in trouble."

An FDA review process for cells refashioned to detect disease, not produce new compounds within the human body, most likely will be faster and less expensive than one for a medical treatment. Many emerging synthetic biology projects are aimed at repurposing bacteria to diagnose the earliest presence of an ailment. "Gut bacteria can be engineered to sense, remember and report on their experiences as they pass through the intestine," says Pamela Silver, a founding member of Harvard's department of systems biology. Silver's lab has created a proof-of-principle diagnostic tool composed of a genetic circuit that enables bacteria to identify the presence of an antibiotic in the digestive system of mice. The circuit produces a fluorescent signal that is visible in fecal waste if the antibiotic is active.

"This synthetic circuit demonstrates our ability to build a living diagnostic—in this case, exposure to antibiotic," Silver says. More recently, her lab has programmed a natural gut bacterium to diagnose gut inflammation and reveal new treatment targets. "The human intestine is a 'dark' place—difficult to explore yet the site of much activity affecting daily health and debilitating diseases, inflammation being one of the most prevalent," she says. Current diagnostics for digestive ills are invasive and costly.

A living diagnostic, Silver says, offers a cheap, potentially more sensitive approach. And if it passes muster, new functions can be added. "We also believe the diagnostic circuits can be further engineered to deliver treatment for bowel disease at the site of inflammation," she says. "The power of the new circuits is creating all manner of possibilities." SA

Journalist **Michael Waldholz** led a team of reporters who were awarded a Pulitzer Prize in 1997 for their coverage of AIDS. He lives in New York State's Hudson Valley.

MORE TO EXPLORE

Engineering Life. Caleb J. Bashor, Timothy K. Lu and Ahmad S. Khalil in *The Scientist,* Vol. 27, No. 8; August 1, 2013. www.the-scientist.com/?articles.view/articleNo/ 36724/ title/Engineering-Life

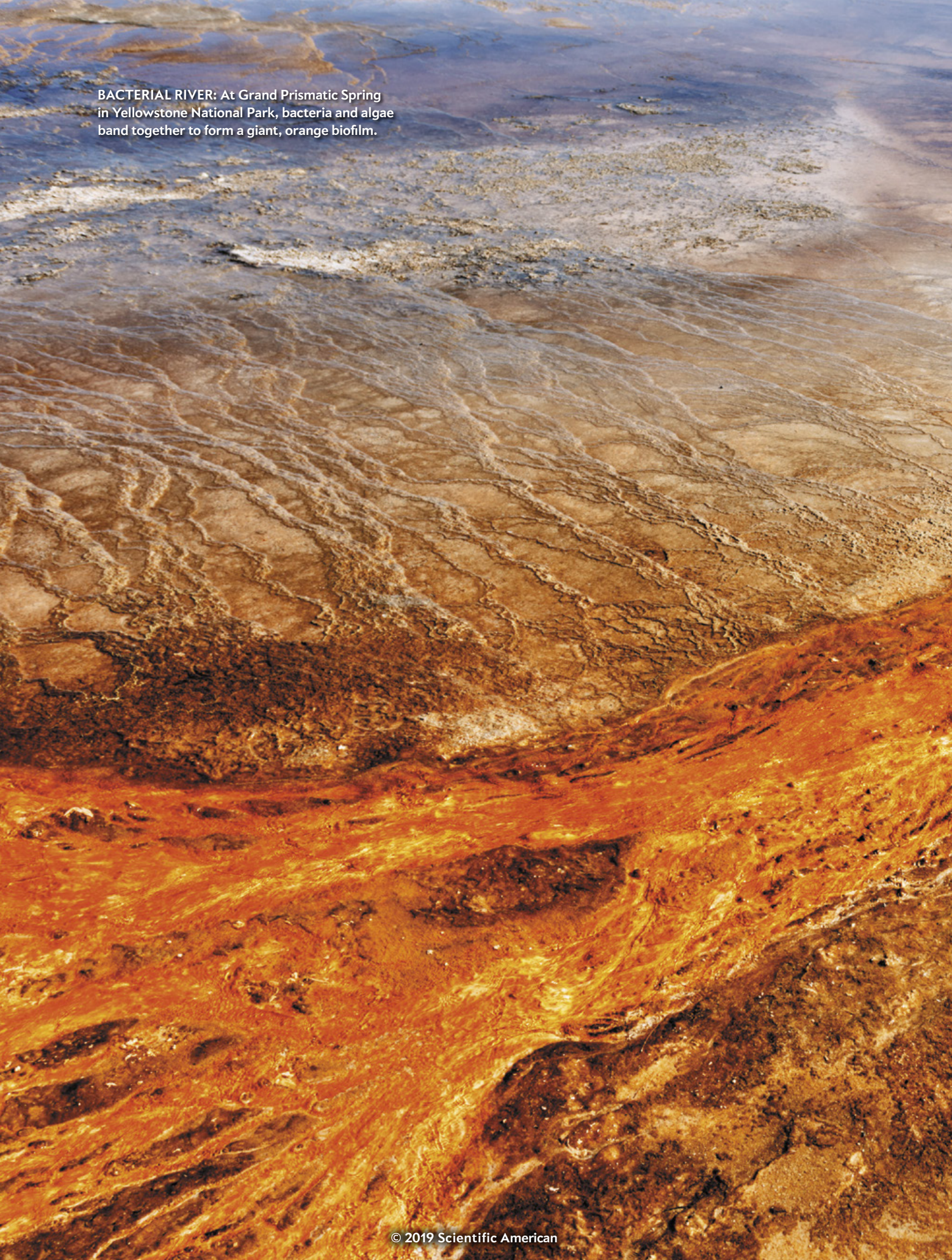
The Era of Synthetic Biology and Genome Engineering: Where No Man Has Gone Before. Timothy K. Lu et al. in *Journal of Molecular Biology,* Vol. 428, No. 5, Part B, pages 835–836; February 27, 2016.

Engineering Bacteria for Diagnostic and Therapeutic Applications. D. T. Riglar and P. A. Silver in *Nature Reviews Microbiology,* Vol. 16, No. 4, pages 214–225; April 2018.

An Engineered *E. coli* Nissle Improves Hyperammonemia and Survival in Mice and Shows Dose-Dependent Exposure in Healthy Humans. Caroline B. Kurtz et al. in *Science Translational Medicine,* Vol. 11, No. 475, Article eaau7975; January 16, 2019.

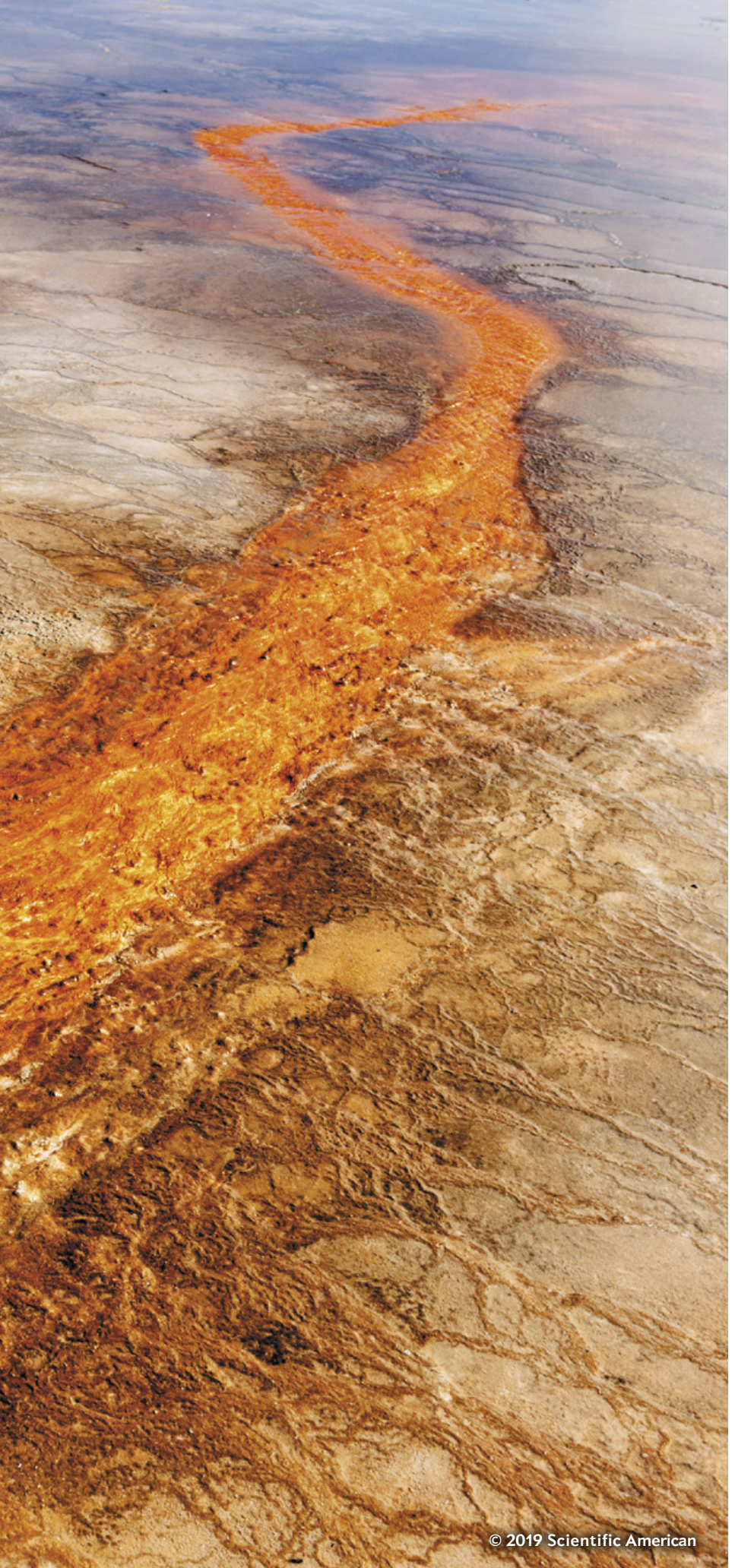
scientificamerican.com/magazine/sa

© 2019 Scientific American



BACTERIAL RIVER: At Grand Prismatic Spring in Yellowstone National Park, bacteria and algae band together to form a giant, orange biofilm.

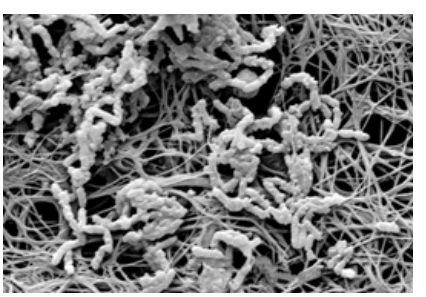
© 2019 Scientific American



Biofilms—3-D mats of bacteria—kill as many people as cancer does and fight off antibiotics. Now scientists are turning biofilms' own weapons against them

By Karin Sauer

THE WAR ON SLIME



MICRO MENACE: The insides of a medical catheter are covered with a bacterial biofilm (*micrograph*) that can cause blood infections.

© 2019 Scientific American

I love Yellowstone National Park.

I have visited countries as far east as Japan, followed the footsteps of Romans, looked up at the Leaning Tower of Pisa, experienced volcanoes from afar and close up, and touched glaciers. Yet I return to Yellowstone again and again, gazing at waterfalls and lakes but especially at the vivid rainbow colors of many of the park's hot springs, geysers, mud pots and fumaroles.

These colors draw me in. They are produced by millions and millions of tightly packed bacterial cells enmeshed in a slimy matrix. Although individual bacteria are not visible to the naked eye, in this slime they form easily seen communities referred to as microbial mats, or "biofilms." Through a microscope these films show remarkable three-dimensional structure, with microbes glued onto one another to form many levels of filaments, winding pathways and features resembling tiny towers. To me, they look like cities of slime, a pulsing metropolis with blocks and skyscrapers and streets that are busier than major avenues in Tokyo or New York. And you've seen biofilms, too: they form the thick and slimy buildup in your drains and the stubborn rings around your bathtub.

Although this buildup is a nuisance, in medicine biofilms are a dire threat to our health. When bacteria succeed in forming a biofilm within people, they resist antibiotics and can be the culprits in chronic infections of surgical sites, lungs and urinary tracts. Biofilms can colonize medical devices and implants such as catheters, prosthetic joints and heart valves. Overall, 65 percent of hospital-acquired infections are caused by bacteria growing as biofilms. There are 1.7 million of these infections annually in U.S. hospitals alone and 99,000 associated deaths, so that is a tremendous amount of harm. Biofilms are thought to claim as many lives as cancer every year—a grim statistic indeed.

Why are biofilms such a problem in medicine? Conventional therapies, geared toward treating infections by single bacteria, have proved inadequate in the treatment of many (if not most) biofilm infections. This is because bacteria growing as biofilms not only have an extreme capacity for evading our host defenses and immune responses but also demonstrate an extraordinary tolerance to antibiotics. This resilience of biofilms is not linked to bacteria having acquired drug resistance or becoming the "superbugs" that we hear so much about these days. Instead it is the 3-D structure that makes biofilms so hardy and difficult to eliminate. Within it, bacteria communicate, cooperate and exchange material to not only organize their architectural features but manufacture proteins and other substances to support and protect one another.

There were early clues that bacteria within biofilms become fundamentally different from single cells. In 1998 researchers George A. O'Toole and Roberto Kolter demonstrated that biofilm formation by the soil bacterium *Pseudomonas fluorescens* required the synthesis of new proteins as well as the presence of 24 genes. Most of the genes were of unknown function, although some encoded proteins used for surface attachment, such as adhesins. The mystery genes suggested that becoming an attached cell meant taking on a novel bacterial physiology. Then, in 2002, my colleagues and I demonstrated that bacteria not only change on surface contact but keep transforming and adapting as the biofilm develops from just a few attached cells into a 3-D community, producing different sets of proteins at each stage. Further work showed that the proteins enable the transition from one biofilm stage to the next in a set sequence.

These findings suggested that biofilms, like cities, are built brick by brick, with their construction following a master plan, one building phase and one city block at a time. Knowing how biofilms are built means that we have started to understand how to interfere with the master plan. In the laboratory, by adding chemicals that inhibit or enhance some of these proteins, we can stop biofilms at a particular developmental stage or even remodel them, making them revert to earlier stages. And some strategies are making their way to the clinic.

IN BRIEF

Biofilms form when groups of bacteria encase themselves in a slime matrix that blocks antibiotics and toxins.

Biologists have figured out some chemical cues microbes use to signal one another to disperse from films.

Dispersion leaves bacteria vulnerable to antimicrobial treatments and can be combined with other tactics.

New nanomaterials can now be engineered to retard biofilm attachment in the first place.

PRECEDING PAGES: GETTY IMAGES

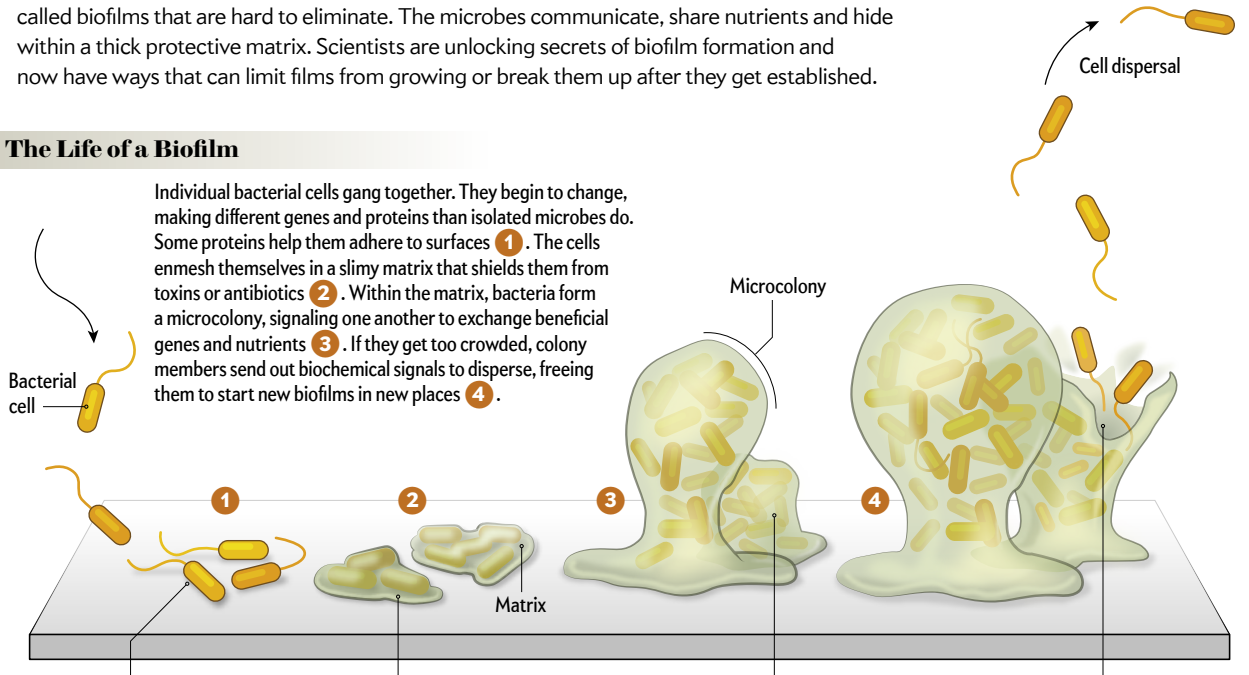
© 2019 Scientific American

Making—and Destroying—Biofilms

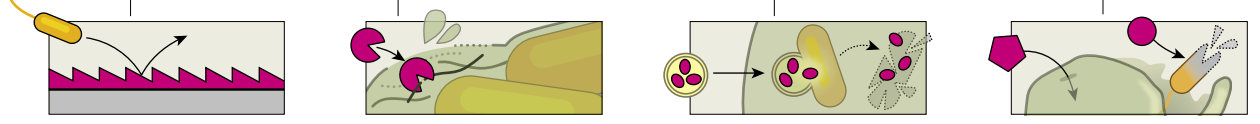
Bacteria are not loners. Most thrive in large groups: complex, mutually supportive communities called biofilms that are hard to eliminate. The microbes communicate, share nutrients and hide within a thick protective matrix. Scientists are unlocking secrets of biofilm formation and now have ways that can limit films from growing or break them up after they get established.

The Life of a Biofilm

Individual bacterial cells gang together. They begin to change, making different genes and proteins than isolated microbes do. Some proteins help them adhere to surfaces 1. The cells enmesh themselves in a slimy matrix that shields them from toxins or antibiotics 2. Within the matrix, bacteria form a microcolony, signaling one another to exchange beneficial genes and nutrients 3. If they get too crowded, colony members send out biochemical signals to disperse, freeing them to start new biofilms in new places 4.



Four Antibiofilm Tactics



A New materials make it harder for biofilms to grab onto surfaces. These materials are made deliberately rough at the nanometer scale, with structures that interfere with proteins that bacteria use for attachment.

B Weakening the matrix can cause structural collapse of the biofilm. The slime contains DNA molecules, which can be broken apart by an enzyme called DNAse I. It is being tested on biofilms that cause ear infections.

C Hollow spheres called liposomes, ferrying antimicrobial compounds, can sink into the matrix. They meld with bacteria, releasing their toxic cargo. Bacteria-infecting viruses can also be inserted into the matrix.

D Biofilm bacteria use a specific fatty acid to signal one another that it is time to leave. In the open, microbes are vulnerable, so scientists are using the acid to trick bacteria into departing. The gas nitric oxide can be used as a similar signal.

AN UNHEALTHY ATTACHMENT

PREVENTING BACTERIAL ATTACHMENT in the first place is a good place to start. Much of this research has focused on the development of surface materials or coatings capable of killing bacteria on contact. That can be accomplished using surface coatings or impregnated surfaces that enable local delivery of antibacterial agents at high concentrations. Several such surfaces are already commonplace in hospitals and include antibiotic-impregnated sutures and bone cement containing antibiotic-impregnated beads, as well as catheters, wound dressings, and endotracheal tubes coated with colloidal silver or silver nanoparticles. The silver ions kill bacteria on contact. The killing mechanism is not fully understood, but we do know that silver ions cause oxidative damage (where oxygen atoms pull electrons away from a microorganism's essential biomolecules), leading to bacterial cell death. In addition to silver, metal oxide and metal salts—iron, mercury, tellurium, zinc, titanium—are being tested for clinical purposes.

Surface coatings and impregnated surfaces, however, have an Achilles' heel because the antimicrobial substance in the coatings eventually run out. Combined with the concerns of overuse of drugs and compounds that are aimed at killing bacteria (including silver) and the consequent emergence of bacterial resistance, this has spawned the development of new surface materials that control attachment in more mechanical ways. The surfaces are inspired by nature, emulating the texture of shark skin, or self-cleaning textures found in lotus leaves, or the chemical functions used by mussels to repel bacteria. Bioinspired surfaces do not necessarily prevent bacteria from attaching but instead interfere with the proteins they use as attachment platforms. This process works by changing, at a microscopic level, surface roughness. That can be done by adding nanostructures such as brushes, crystals and tubules made of polymers that are attracted to water

Illustration by Jen Christiansen

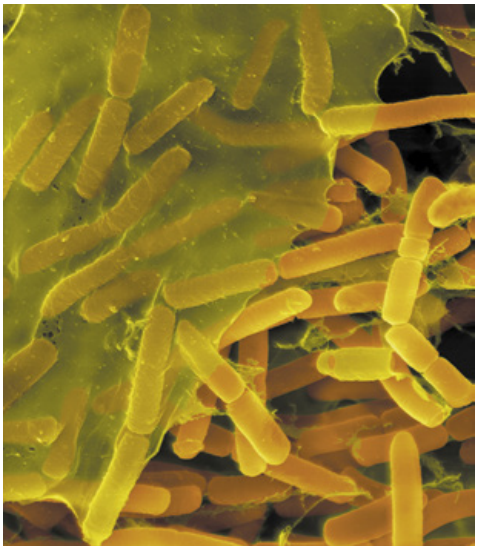
© 2019 Scientific American

molecules, such as polyethylene glycol (PEG, inspired by mussels), and compounds known as zwitterionic polymers that were inspired by the antibiofouling properties of blood cells.

The nanostructures can be arranged at varying distances to reduce bacterial attachment to different degrees. Some of these bioinspired surfaces have now been widely used in clinics. Others are still at the proof-of-concept stage, with hurdles such as manufacturing limitation and toxicity issues to be overcome.

ATTACK THE MATRIX

DESPITE THE PROMISE of antibiofilm surfaces for medical implants, very different strategies are needed to treat biofilms that overcome such antiattachment strategies and form in dangerous places (in the absence of man-made surfaces), such as in wounds or lungs. In such cases, the slimy matrix surrounding biofilms becomes the target. This matrix, composed of long strands of sugar molecules called polysaccharides, proteins and DNA, helps the sheets of microbes in several ways. For one, the matrix functions as a protective layer. It blocks or restricts some antibiotics or immune system markers known as antibodies from reaching the bacteria within the biofilm structure. In addition, the matrix serves as a structural framework that glues the bacterial community together and to the surface. Thus, removal of this framework can result in loss of structural integrity and subsequent collapse, as the large bacterial gang breaks up into smaller cell clusters or individual cells. When that happens, the liberated bacterial cells become vulnerable once again to drugs and the immune system.



TOGETHERNESS: ***Bacillus*** **bacteria (*oblong shapes in micrograph*) cover themselves in a protective matrix.**

The downside of targeting the structural framework is that the composition of the biofilm matrix, more specifically the type of proteins and polysaccharides present in the matrix, varies greatly among bacteria, with each type requiring a specific degradative enzyme. Therefore, treatments aimed at the degradation of proteins and polysaccharides need to be tailored to the type of bacterium that forms the biofilm. Recent findings by Kendra Rumbaugh of Texas Tech University and colleagues suggests that this hurdle may be overcome using glycoside hydrolases, enzymes that target common, highly conserved glycosidic linkages present in polysaccharides. The team demonstrated that glycoside hydrolases effectively disrupt *Staphylococcus aureus* and *Pseudomonas aeruginosa* monoculture and coculture biofilms, leaving the disintegrated biofilm cells more susceptible to conventional antimicrobials. Follow-up tests in animals suggest that such therapies could potentially prove effective against a wide range of pathogens with diverse matrix compositions.

Another common matrix component may be DNA that appears to be universally present in biofilms. The matrix DNA is susceptible to degradation by one enzyme alone: DNAse I. Several clinical trials are focusing on the use of this substance; it is being evaluated for the treatment of chronic middle ear (otitis media) and other biofilm infections in combination with antibiotics. DNAse I is already used in the treatment of cystic fibrosis patients with early lung disease, with treatment coinciding with significantly improved lung function. (In this case, however, the enzyme seems to be reducing the stickiness of the sputum, thus enhancing lung clearance and antibiotic efficacy rather than inducing a collapse of the biofilms.)

SOUND THE RETREAT

A STRATEGY OF A DIFFERENT nature has been gleaned from watching the way in which biofilms develop. After bacteria form these films, they can disassemble them via a process called dispersion. Dispersion enables biofilms to revert to a single cell state and occurs when resources within them, such as nutrients, become exhausted, or when biofilms become too overcrowded, or when the outside environment becomes unstable. Breaking up can help biofilm members survive and spawn new communities at other locations.

Single cells leaving a biofilm are, however, exactly the kinds of unprotected bacteria we are good at treating with medicine. So how can we redirect film-forming bacteria to switch their lifestyle and escape from the biofilm? Researchers have identified several environmental growth conditions and compounds capable of inducing the switch, including exposure to heavy metals, availability of nutrients and oxygen, and presence of signaling molecules such as nitric oxide and a small fatty acid molecule named *cis*-2-decenoic acid (*cis*-DA). While these dispersion triggers are quite diverse, they all have in common that they induce dispersion by lowering the level of a universal intracellular signaling molecule named cyclic di-GMP. Levels of cyclic di-GMP determine the stickiness of bacteria to surfaces, with high levels being linked to surface-associated biofilm growth and matrix production and low levels being linked to bacteria growing as single cells. Although changes to cyclic di-GMP levels in response to these triggers have been linked with 80 percent or more of the biofilm biomass being removed, not all dispersion triggers are suitable for use in medical settings.

One viable candidate to emerge is the colorless gas nitric oxide. Our own immune system makes use of nitric oxide to fend off bacterial invaders, and it is already used medically to improve oxygenation in patients who have various forms of pulmonary hypertension (for example, chronic obstructive pulmonary disease). Lab studies have shown that nitric oxide mediates biofilm dispersal across a broad range of bacteria. Nitric oxide alone reduces biofilms on average by 63 percent. When com-

DENNIS KUNKEL *Science Source*

© 2019 Scientific American

bined with antimicrobial compounds such as colistin, the oxide was able to almost completely remove biofilms in lab experiments.

Still, despite the promising results, nitric oxide poses some clinical challenges. It can be toxic if it spreads to different areas of the body, so delivery and restriction to a specific site of infection are important—and hard to do with a gas. To better address some of the concerns, various formulations and devices have been developed. One example is cephalosporin-3′-diazeniumdiolates. This combination drug is composed of an antibiotic (cephalosporin) and a nitric oxide–producing substance that activates only on contact with bacterial cells that harbor an enzyme called β-lactamase. Bacteria resistant to antibiotics such as penicillin and ampicillin typically have this enzyme.

Research on *P. aeruginosa,* a model bacterium for biofilm formation and the cause of a large number of hospital-acquired and chronic infections, has shown that bacteria have their own way of telling members in the biofilm community to disperse. This bacterium produces *cis*-DA, which enables the organism to signal to its community members that it is time to leave. The ability of *cis*-DA to signal dispersion is not limited to *P. aeruginosa* biofilms. Instead *cis*-DA has been found to signal biofilms formed by other bacteria as well as yeast—not *P. aeruginosa*—to break up. There is also evidence that other bacteria produce a different version of this fatty acid, which means that biofilm bacteria may use particular "dialects" to tell members to disperse. Though still at the lab stage of testing, using such signaling and communication molecules in combination with antibiotics may very likely represent a future treatment strategy.

A recent study furthermore suggested the availability of pyruvate, a key intermediate in several metabolic pathways, to act as a toggle switch between the planktonic and biofilm lifestyle—high levels of pyruvate seemingly enhancing biofilm formation and depleted pyruvate inducing dispersion. In lab experiments, researchers used enzymes to deplete pyruvate, resulting in dispersion by the two pathogens *P. aeruginosa* and *S. aureus*. Moreover, when combined with antimicrobial compounds, such pyruvate-depleting conditions enhanced the potential of antibiotics such as tobramycin in killing biofilms. The combination strategy was also found to be effective in animal studies when tested on burn wounds infected with *P. aeruginosa*. In fact, the combination treatment was found to be more effective in eradicating biofilms present in burn wounds than the more commonly used antibacterial cream silver sulfadiazine.

DETECT AND INFECT

ANOTHER STRATEGY to combat biofilms is to cause a lethal infection of their bacterial constituents. Like people, bacteria are susceptible to viruses, which in the case of these microbes are referred to as phages. Yet while people are not affected by phages, bacteria will be either permanently infected or killed. Since early studies in 1996 examining the interaction of phages with biofilms, research has aimed at identifying phages capable of killing bacteria within a biofilm. Moreover, phages that enter but do not kill bacterial cells can still be used as cargo trucks to deliver an antibiotic or a matrix-degrading agent (such as DNAse I) to each and every bacterium within the biofilm. Phage therapy is currently being used outside the U.S. in the treatment of biofilm-derived lung infections by cystic fibrosis patients.

But the therapy, like other tactics, comes with its own set of problems. Because phages infect and kill bacteria with high specificity, a particular phage infects only a specific type of bacterium, meaning they cannot be used as broad-spectrum killers. And bacteria can become resistant to phage attack, just like they develop resistance to antibiotics.

To overcome potential resistance, researchers have developed liposomes, or lipid-enclosed vesicles, as alternative cargo carriers. Referred to as nanoparticles because of their small size, the idea behind these mini transporters is to deliver compounds that kill biofilm cells or destabilize biofilms such as antibiotics, antibacterial compounds or matrix-degrading enzymes directly to where they are needed, into the biofilm or to the bacteria themselves. The targeting mechanism is based on lipids in the liposomes that mimic the lipids on bacterial cell membranes. The similarity allows them to diffuse into the matrix. Here lies the beauty of the system. The matching lipids enable liposomes to fuse with a bacterium and thus enable the transport system to directly spill its cargo into the microbe in a manner similar to an injection. An advantage of this strategy is the targeted approach because antibacterial and antibiofilm compounds are delivered only to the biofilm and nowhere else. Liposomes are already a very widely used antimicrobial drug-delivery system.

WINNING COMBINATION

THE TACTICS DESCRIBED HERE barely scratch the surface of the sheer number of strategies that are being tested in labs worldwide, some at early stages of development, some at the preclinical stage. But these efforts not only indicate the importance of biofilm control but also how challenging it is to truly get a handle on slime, mainly because all biofilms are not the same. Each bacterial species makes and escapes from biofilms in slightly different ways, using variations of communication molecules, producing different proteins and more. Yet as the approaches indicate, we are getting closer to a treatment that can help people, although such treatment may take advantage of a combination of antibiofilm strategies to demolish the bacterial cities that threaten our lives. SA

Karin Sauer is a biology professor at Binghamton University, State University of New York. She is co-director of the Binghamton Biofilm Research Center, where she researches the mechanisms underlying biofilm development, dispersion and antimicrobial resistance.

MORE TO EXPLORE

Cephalosporin-3′-diazeniumdiolates: Targeted NO-Donor Prodrugs for Dispersing Bacterial Biofilms. Nicolas Barraud et al. in *Angewandte Chemie International Edition,* Vol. 51, No. 36, pages 9057–9060; September 3, 2012.

Re-Establishing a Place for Phage Therapy in Western Medicine. E. M. Kutter, S. J. Kuhl and S. T. Abedon in *Future Microbiology,* Vol. 10, No. 5, pages 685–688; May 2015.

Control of Biofilms with the Fatty Acid Signaling Molecule cis-2-Decenoic Acid. C. N. Marques, D. G. Davies and K. Sauer in *Pharmaceuticals* (Basel), Vol. 8, No. 4, pages 816–835; November 25, 2015.

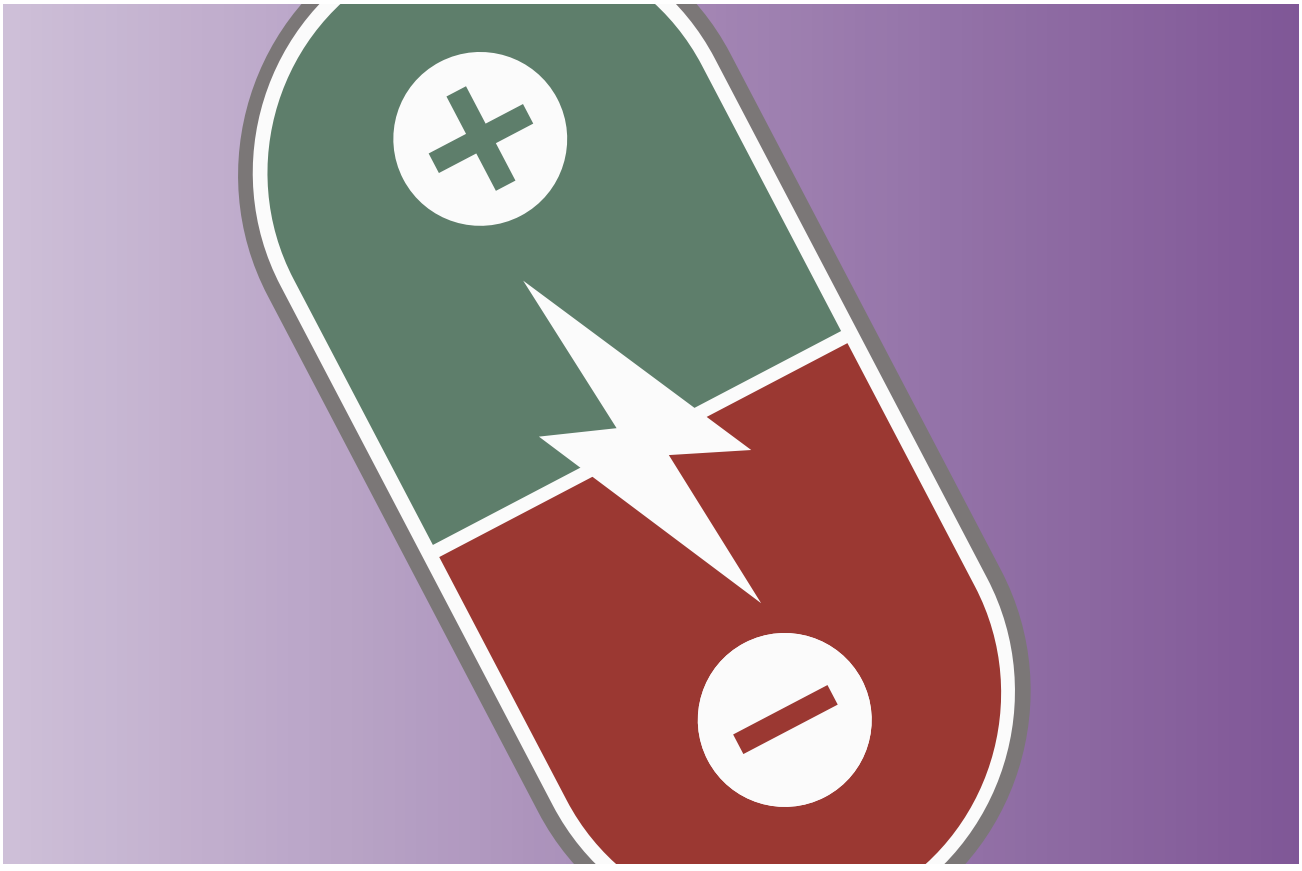
Bactericidal, Quorum Quenching and Anti-Biofilm Nanofactories: A New Niche for Nanotechnologists. B. N. Singh et al. in *Critical Reviews in Biotechnology,* Vol. 37, No. 4, pages 525–540; June 2017.

The Consequences of Biofilm Dispersal on the Host. Derek Fleming and Kendra Rumbaugh in *Scientific Reports,* Vol. 8, Article No. 10738; July 16, 2018.

Pyruvate-Depleting Conditions Induce Biofilm Dispersion and Enhance the Efficacy of Antibiotics in Killing Biofilms in Vitro and in Vivo. James Goodwine et al. in *Scientific Reports,* Vol. 9, Article No. 3763; March 6, 2019.

scientificamerican.com/magazine/sa

© 2019 Scientific American



Electroceuticals

Nerve-stimulating therapies could soon replace drugs for many chronic conditions

By Geoffrey Ling and Corinna E. Lathan

ELECTROCEUTICALS—DEVICES that treat ailments with electrical impulses—have a long history in medicine. Think pacemakers for the heart, cochlear implants for the ears and deep-brain stimulation for Parkinson's disease. One of these approaches is poised to become more versatile, dramatically improving care for a host of conditions. It involves delivering signals to the vagus nerve, which sends impulses from the brain stem to most organs and back again.

New uses of vagal nerve stimulation (VNS) have become possible in part because of research by Kevin J. Tracey of the Feinstein Institutes for Medical Research and others showing that the vagus nerve emits chemicals that help to regulate the immune system. The release of a specific neurotransmitter in the spleen, for instance, quiets immune cells involved in inflammation throughout the body. These findings indicated that VNS might be beneficial for disorders beyond ones that are marked by disturbed electrical signaling, such as autoimmune and inflammatory conditions.

VNS could be a boon for patients with those conditions because existing drugs often fail or cause serious side effects. It may be easier to tolerate because it acts on a specific nerve, whereas drugs generally travel throughout the body, potentially disrupting tissues beyond those targeted for treatment.

So far studies of inflammation-related applications are encouraging. VNS devices developed by SetPoint Medical (cofounded by Tracey) have proved safe in early human trials for rheumatoid arthritis, which causes painful, disfiguring inflammation of joints, and for Crohn's disease, which involves inflammation of the intestines. Additional trials for both are currently underway.

The electroceutical approach is also being considered for other maladies that have an inflammatory component, such as cardiovascular disease, metabolic dysregulation and dementia, as well as for autoimmune disorders such as lupus, in which the vagus nerve itself becomes underactive. Preventing immune rejection of transplanted tissues is yet another potential application.

Most vagal nerve stimulators, including SetPoint's devices and those already in use for treating epilepsy and depression, are implants. Physicians usually place the device under the skin below the clavicle. Wires from the implant wrap around one branch of the vagus nerve and deliver electrical impulses to it at preset

ROBERT GALE *World Economic Forum*

© 2019 Scientific American

intervals; the frequency and other properties are programmed via an external magnetic wand. Today's implants measure about an inch and a half in diameter but are expected to become smaller and more finely programmable over time.

Noninvasive, handheld vagal nerve stimulators meant to ease cluster headaches and migraine have recently also gained approval from the Food and Drug Administration, although exactly how vagal nerve stimulation helps those conditions is unclear. The handheld devices deliver mild electrical stimulation to the nerve through skin on the neck or through the ear.

The vagus nerve is not the only one to be targeted by new electroceutical approaches. In late 2017 the FDA approved a nonimplanted device that eases opioid withdrawal by sending signals to branches of the cranial and occipital nerves through skin behind the ear. The device gained the FDA's nod after 73 patients suffering from opioid withdrawal saw a 31 percent or higher reduction in symptom severity.

The cost of implants and surgery could hamper widespread adoption of VNS therapy, although that problem should ease as the technology becomes less invasive. But cost is not the only challenge. Researchers still need to know more about how VNS produces its effects in each condition and how best to determine the optimal patterns of stimulation for individual patients. It is also possible that impulses targeted at the vagus nerve may affect surrounding nerves in undesirable ways.

Nevertheless, as more studies and trials examine their mechanisms and effects, vagal nerve stimulators and other electroceuticals may ultimately be able to better manage a wide range of chronic disorders, potentially reducing the need to take medicine for millions of patients. SA

Geoffrey Ling, a retired U.S. Army colonel, is a professor of neurology at the Uniformed Services University of Health Science and at Johns Hopkins University. An expert in technology development and commercial transition, he has held leadership positions at the Defense Advanced Research Projects Agency and, under President Barack Obama, in the White House Office of Science, Technology and Policy.

Corinna E. Lathan is co-founder and chief executive officer of AnthroTronix, a biomedical engineering research and development company creating products in digital health, wearable technology, robotics and augmented reality. She is also on the board of PTC, a provider of Internet of Things and augmented-reality platforms.

Bacterial Tape Recorder

Researchers program bacteria to record cellular memories

By Yasemin Saplakoglu

CRISPR IS BEST KNOWN AS being the basis of a powerful gene-editing tool. But first and foremost, it is a defense that bacteria use against viruses. Inspired by this delicate natural system, researchers have now created another scientific application for it—a tiny "tape recorder" that chronicles biological signals on strands of a bacterium's DNA.

The investigators believe this microbial recorder could eventually be used for sensing abnormalities in bodily functions such as digestion, for measuring pollutant levels in oceans or for detecting nutrient changes in soil. It works much like the natural CRISPR system in many bacteria and other single-cell organisms, except for the signals it detects.

CRISPR is a DNA sequence that makes and keeps a genetic record of viruses the bacterium encounters, commanding it to kill any that try to reinfect the bacterium or its descendants. Whereas the natural CRISPR system remembers viral DNA, the new application can track a variety of biochemical signals. For example, these bacterial recorders could detect the presence of the sugar fucose in a human's gut, indicating an infection.

When the bacterium senses a specified signal, it creates many copies of what is called trigger DNA, which then get recorded on one end of its genetic "tape." The tape continues to record in the absence of the designated signal, registering the "background noise" of other pieces of DNA sloshing around in the cells. These background signals serve as time stamps on the recordings. Scientists at Columbia University reported the findings in a study published in December 2017 in the journal *Science*.

The researchers suggest that a few million bacteria that are outfitted with copies of this tool could be deployed in the human body or the environment, where they would passively record until they are recovered from feces or soil samples and the tapes can be read. Unlike most previous biological memory systems, this one is fully under the bacterial cells' control.

"The DNA is writing itself in response to changes in the environment, whereas in the prior work you sort of had a puppeteer showing that the DNA could be written—but somebody was pulling the strings," says Drew Endy, a synthetic biologist at Stanford University, who was not part of the study.

Although this technique has been tested only in the laboratory, the team showed it could continuously record three different signals in a population of *Escherichia coli* cells for three consecutive days.

Recording ability decreased with time, probably because operating as a tape recorder does not confer any survival benefits, Endy says. He also notes that the signal needs to be present for six hours for the tool to reliably record it, which may be too long to detect fleeting signals. Harris Wang, a synthetic biologist at Columbia and senior researcher on the study, hopes to speed that process up in future work. SA

Yasemin Saplakoglu is a science writer based in New York City. She is a staff writer at Live Science and was an editorial intern at *Scientific American*.

© 2019 Scientific American

© 2019 Scientific American

THE MEANS OF REPRODUCTION

Could scientists one day use blood and skin cells to replace sperm and eggs?

By Karen Weintraub

Illustration by Raul Arias

© 2019 Scientific American

THE MICE SCURRYING AROUND THEIR CAGE IN KATSUHIKO HAYASHI'S LABORATORY do not look remarkable. They run, eat and sleep like others of their kind. But these eight rodents have an unusual origin story, one that Hayashi, a reproductive biologist at Kyushu University in Japan, revealed three years ago in the pages of *Nature*. The tawny-colored mice, he and his colleagues announced, did not spring from the mating of sperm and egg. On their mother's side, their roots trace to a reprogrammed skin cell.

The advance, called "amazing" by other researchers, delivers on a promise hinted at in 1997, when scientists managed to clone Dolly the sheep. That accomplishment built on earlier cloning work in frogs from the 1970s and taught scientists that every animal cell has the same basic set of instructions. By transforming a sheep's mammary cell into a living animal, Dolly's creators showed that every mammalian cell has the same genes—and that the difference between a breast cell and any other cell is simply which genes are turned on or off.

For Hayashi and other scientists, that work created the prospect that they might be able to reprogram mammalian cells to become anything from a neuron to an egg if only they could devise the right instruction manual. A small number of researchers around the world, including Hayashi, are using this legacy to tackle in vitro gametogenesis—generating eggs and sperm from adult cells.

Reproductive scientists and some couples struggling with infertility are closely tracking Hayashi's progress, as well as similar efforts that have successfully converted rodent stem cells (progenitor cells that can develop into any type of specialized cell) into rudimentary sperm. If these egg and sperm techniques can be made to work in humans, we may one day be able to replace our faulty gametes with blood or skin cells. In that future, men would not have to worry about a lack of healthy sperm. And instead of watching their chances of motherhood fade with their 30s, women of virtually any age could give a little blood and end up with a batch of eggs. Gay couples, too, might one day be able to have children to whom they are both biologically related.

The hope remains tantalizing but distant. Years of animal experiments aimed at finding a reliable substitute for the egg and sperm cells essential to creating most mammalian life have not yet succeeded. But even this very preliminary work in mice and human cells is prompting a wave of ethical questions from the scientific community about eventual human applications.

PLANNING PARENTHOOD

TO MAKE THIS REPRODUCTIVE PROCESS WORK in mice, Hayashi's team needed to tie together several earlier discoveries. In 2010 it practiced hitting the "reset" button on cells, sending them back to a stage before they had found their identity. The team began by retracing a process developed by Shinya Yamanaka of Kyoto University in Japan, for which he won a Nobel Prize in 2012.

First, the researchers scraped skin cells off an adult mouse's tail. They then injected them with a chemical cocktail containing four specific genes to transform adult cells back into stem cells capable of becoming many different kinds of cells. Next, they employed genetic insights established in the early 2000s by Azim Surani of what is now the Gurdon Institute in England and Mitinori Saitou, who was then working in Surani's lab. (Both men would later mentor Hayashi.) This work, and related experiments with embryonic cells derived from regular mouse embryos, eventually helped Hayashi's team understand which genes would be needed to coax stem cells into becoming egg progenitor cells called primordial germ cells.

There was a catch: primordial germ cells, which can develop into either sperm or eggs, still have two sets of chromosomes like any typical animal cell. To form sex cells, which have just one set of genes from each parent, germ cells must twice undergo cell division in a process called meiosis. In females, the first cell division happens in the embryo as the primordial germ cell enters the reproductive system. The second division happens during ovulation when the egg is finally matured after exposure to a number of hormones. After creating the primordial germ cell, Hayashi and his co-workers were able to place them back into a live mouse to com-

IN BRIEF

Mammals typically require eggs and sperm for reproduction.
Recently scientists have managed to convert skin stem cells into viable eggs in mice.
In 2016 researchers created eight healthy mice by coupling eggs derived from skin stem cells with standard mouse sperm.
Scientists hope these accomplishments will eventually lead to more future reproductive options for human infertility.

© 2019 Scientific American

plete their development—reaching what was then the boundary of science. To create viable eggs in a dish, however, researchers would need to understand and re-create each step along the pathway to maturation.

The key, the scientists discovered, was to more carefully mimic nature. They spent several years tweaking the solution in which the converted egg cells were grown. One breakthrough came when the team added cells from ovaries of other mouse fetuses into the medium while they matured the cells in a dish. The ovaries released a mixture of hormones—basically creating an ovarylike environment to fool the cells into thinking they were in the body. Furthermore, the scientists altered the viscosity of the fluid medium to mimic what would be found in the mouse.

Once they got that medium right, and the eggs were matured in the lab, the next steps were akin to any other in vitro fertilization (IVF) procedure. The researchers first married the mature eggs and normal mouse sperm together in the lab. After a few days, they selected a promising embryo using a tiny pipette and injected it into a female mouse that would incubate the mouse fetus for 20 days. Finally, after many failed attempts in which the mouse would miscarry, or the embryo would not implant, or it would become stillborn, the process at last led to one healthy pup. Eventually more followed.

The process is still far from perfect, however. Only 16 of the hundreds of stem cells Hayashi's group created survived the five-week mouse-egg-maturation process. And when the scientists coupled the successful lab-made eggs with sperm, only an extremely small percentage of those skin-cells-turned-eggs went on to become healthy mouse pups (compared with a 62 percent success rate for eggs taken from adult mice and fertilized in vitro). Yet the scientists proved that their methods could work. Those eight pups grew up to be normal and healthy. They even went on to have mates and to birth lively pups of their own.

WHEN SPERM MEETS EGG

PLENTY OF PEOPLE require reproductive help. More than 10 percent of American men and a similar percentage of women are considered infertile. Options for overcoming infertility are arduous and often unsuccessful. IVF, for example, requires a woman to undergo a week or two of hormone shots designed to release multiple eggs. A handful of those eggs will then be fertilized with sperm in a lab, and one or two will be implanted. The cost, largely paid out of pocket, can easily top $20,000, and yet approximately 65 percent of in vitro fertilization cycles still fail, often because of poor egg quality. Moreover, IVF cannot help if someone has no healthy eggs or sperm.

It is obvious why mining human blood or skin cells to make a baby is an alluring alternative. Instead of extracting human eggs, a health care worker could draw a small vial of a potential mother's blood. (Blood, which is routinely drawn in medical facilities, might be easier to access in human patients than skin cells, Hayashi says, although either could be used.)

Scientists in a lab could transform those blood cells into stem cells and then, after a few more steps, into eggs or sperm. Next, the manufactured egg could be fertilized with normal sperm, or vice versa, and implanted into the woman using the same method as IVF—leaving the child with the same genetic

A Recipe for Making Babies from Skin Cells

In pursuit of a workaround for female infertility, Katsuhiko Hayashi of Kyushu University in Japan and his colleagues have been trying to find a way to convert skin cells into viable eggs. After thousands of failed experiments, in the fall of 2016 they managed to find the right chemical milieu and laboratory conditions to reprogram skin cells from mice into viable gametes. Next, they were able to use in vitro fertilization technology to marry those lab-grown eggs with standard sperm. Eight healthy mice have been born as a result of this breakthrough. The insights from this technique, researchers hope, could edge science closer to new options for humans.

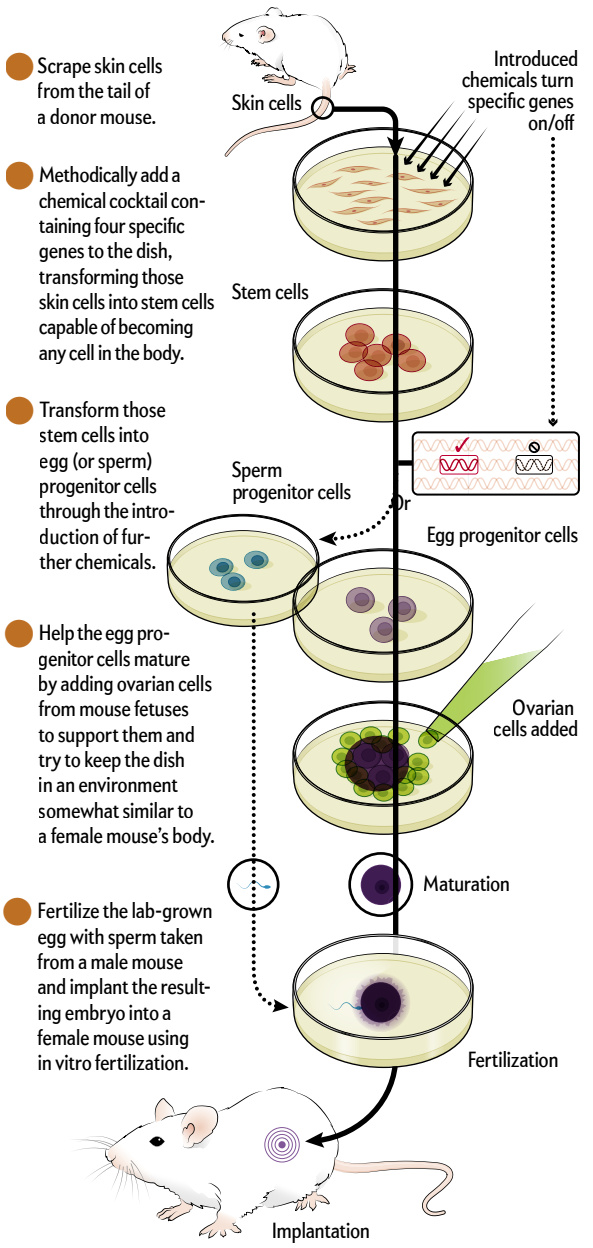

Scrape skin cells from the tail of a donor mouse.
Skin cells
Introduced chemicals turn specific genes on/off
Methodically add a chemical cocktail containing four specific genes to the dish, transforming those skin cells into stem cells capable of becoming any cell in the body.
Stem cells
Transform those stem cells into egg (or sperm) progenitor cells through the introduction of further chemicals.
Sperm progenitor cells
Or
Egg progenitor cells
Help the egg progenitor cells mature by adding ovarian cells from mouse fetuses to support them and try to keep the dish in an environment somewhat similar to a female mouse's body.
Ovarian cells added
Maturation
Fertilize the lab-grown egg with sperm taken from a male mouse and implant the resulting embryo into a female mouse using in vitro fertilization.
Fertilization
Implantation

Illustration by Tami Tolpa

© 2019 Scientific American

inheritance he or she would normally get from each parent.

Hayashi says that right now the procedure is too risky to apply to humans and would become acceptable only if the eggs that scientists create can lead to healthy embryos as often as natural ones do. To begin with, researchers will have to show that they can keep the eggs alive in the lab long enough to closely emulate what would be necessary for human development. (In mice, egg cells mature in five days; in women, they require roughly 30.) Yet before reproductive scientists can even think about creating babies this way, they will have to confirm that the process will work in larger animals that more closely resemble people.

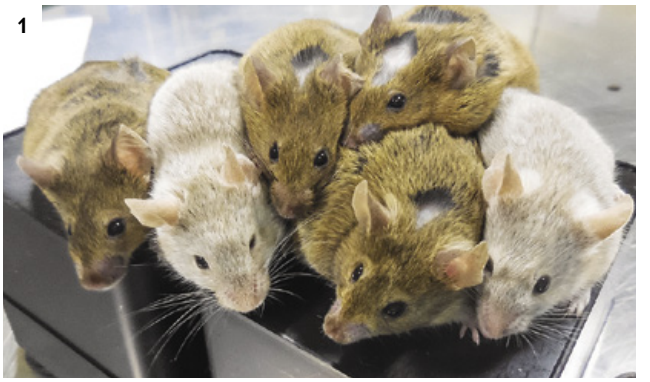
GROWING TOGETHER

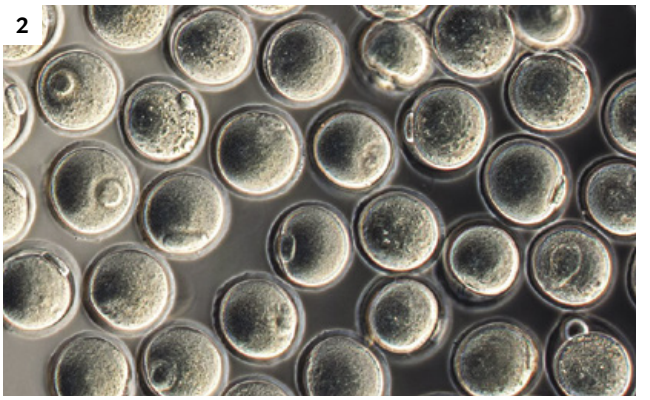
TO OVERCOME THAT HUMP, Hayashi's team is already working with primates: marmoset monkeys. But several major challenges have hindered its progress. Mice are good research subjects because they ovulate every five days and are pregnant for 20. Marmoset pregnancies last more than 140 days, so making a baby would take longer, even if all the science worked perfectly. It takes much longer for primordial germ cells in a marmoset to mature into eggs than it does for mouse eggs to mature, and Hayashi and his team have yet to find a lab environment that keeps the cells alive long enough for that to happen.

In their rodent work, the researchers learned how to mature primordial germ cells outside of living mice, but they still needed ovarian cells from mice fetuses to aid the process. To make sure the primordial germ cells survive and mature in monkeys—and to be able to scale up this work to ultimately create many more lab-grown eggs—Hayashi thinks he will have to do more than simply transfer ovarian cells to a lab dish. He will have to identify the specific ovarian cells that send key signals for maturation and figure out how to derive them from stem cells. That way, in future stages of the work, he would be able to grow all the necessary ingredients—rather than remain dependent on mining other fetuses for their ovarian cells.

Surani, director of germ-line and epigenomics research at Gurdon and a pioneer in this field, has been experimenting with different combinations of these key "helper" cells to support the germ cells' maturation and communication. "The [germ] cells actually go through to a certain point, and then they need something very specific to break through the next point—they need a change of signal or environment—or something," Surani explains. He and his team have been making educated guesses about which cells may be particularly significant in that process, but it is slow, painstaking work. To help guide their next steps, they are now studying aborted human fetuses for clues about each step of egg cell maturation. The lab has also started using pigs, instead of mice, because porcine development more closely resembles that of humans and because pigs are cheaper to work with than monkeys. Saitou and his lab recently published a paper in *Science* showing that they had created human egg precursor cells from adult cells. The process took a long time and yielded few egg-precursor-like structures, suggesting that making eggs in humans might be far more complicated than it was in mice.

Rather than tweaking lab dish protocols, there might be another way to further the process. Some researchers think they will get better results by moving their manufactured cells in vivo as soon as possible to piggyback off the body's natural quality-control systems that eliminate flawed gametes and leave more resources for the remaining cellular contenders. Renee Reijo Pera, a stem cell scientist at Montana State University, is taking that tack in her research with sperm. In nature, only the fittest sperm survive to fertilize the egg, but making and maturing sperm in a lab dish does away with that competition, increasing the risk that defective sperm will fertilize, she says. Because the human body is exquisitely tuned to weed out bad sperm, Reijo Pera focuses her work on making primordial sperm that can be matured in the testes. "We think the body should do the selecting," she says. "In a dish, I'm worried we'll force things to go forward that wouldn't in the natural environment."





MICE CREATED from lab-made eggs and typical mouse sperm are healthy (*1*). These eggs were derived from embryonic stem cells (*2*).

KATSUHIKO HAYASHI *Kyushu University*; FROM "MOUSE EGGS MADE FROM SKIN CELLS IN A DISH," BY DAVID CYRANOSKI, IN *NATURE*, VOL. 538; OCTOBER 20, 2016 (*1, 2*)

No matter what precautions scientists take, some critics say artificial eggs or sperm should never be used to create human life. Marcy Darnovsky, for example, does not think that lab-generated germ cells could ever be safe enough to justify their risks. Darnovsky is executive director of the Center for Genetics and Society, a public-interest organization advocating for the responsible use of human genetic technologies. She says she fully supports research that leads to a better understanding of human and animal development. But she draws a line at using engineered eggs and sperm to generate a new life—especially a human one. "I think it's likely to be extremely biologically risky for any resulting children," she says, citing the example of mammalian cloning: many of the cloned embryos failed to develop, and some animals were born with terrible health problems. Darnovsky believes that public policy is needed to make sure that the scientific progress Hayashi, Surani, Reijo Pera and others are pursuing does not go too far.

© 2019 Scientific American

Other concerns persist about what this methodology might mean for our understanding of parenting. If anyone's cells could be manipulated into becoming sperm or egg, for example, could that portend a future where individuals' cells could become both sperm and egg—creating a uniparent? Or might someone be able to snatch up a stray skin cell from a person's napkin or bed to create a child without his or her consent or knowledge? Moreover, as George Daley, dean of Harvard Medical School, and his colleagues wrote in 2017 in *Science Translational Medicine,* such a technology could enable the creation of embryos on a previously unimagined scale—raising the specter of the devaluation of human life, as well as vexing policy challenges.

Ethical concerns have thus far constrained any human-related work on in vitro gametogenesis and have kept funding to a minimum, researchers say. Science involving embryos has long been restricted in the U.S. Whereas the Obama administration was friendlier to stem cell research than its predecessor, the pendulum has swung back somewhat under Donald Trump. In other countries as well, the lack of funding for such research and difficulty in accessing tissue samples of natural embryos for comparison add an extra layer of challenge to the research, according to Surani and Helen Picton, who does related work at the University of Leeds in England. Hayashi, for example, says it would be very difficult for him to do human germ-cell studies in his native Japan. (Japanese law forbids fertilizing human germ cells, even for research purposes.) But Jacob Hanna, a stem cell scientist at the Weizmann Institute of Science in Israel, says he has an easier time because of cultural interest in advancing reproductive technologies.

AN ETHICAL CONUNDRUM

EVEN IF THEY NEVER produce a human baby, though, scientists say simply pursuing the goal of making eggs and sperm will have payoffs: in treating infertility, understanding early development and unraveling the effect that toxins can have on human inheritance. "It's a voyage of discovery," says Picton, who specializes in ovarian physiology and reproduction. Figuring out how to identify high-quality eggs and sperm may help improve the selection process for IVF, for example. And the process of refining the recipe for gamete creation will provide the first real insights into where cells go wrong to cause disease, birth defects or cellular death.

Learning how to make eggs and sperm from skin or blood cells might also help scientists better unravel genetic inheritance known as epigenetics—changes not to the genes but to gene expression. Understanding how sperm and eggs are formed in their earliest days might allow us to scour those cells for any methyl groups or other changes that have accumulated in the genes. Right now questions abound regarding how some traits seem to get passed down without altering the underlying genetics. In a 2016 study, for instance, epigenetic changes to areas of genes associated with regulating stress hormones were found in the children of Holocaust survivors born years after their parents' trauma. The genes were unchanged, but how the genes acted seemed to get passed down. Being able to generate eggs and sperm from stem cells could allow scientists to dig into this epigenetic process, Surani says, and could offer insights into diseases of aging, which are often caused by the accumulation of epigenetic markers. Treatments for aging-related diseases might even come out of a new understanding of how these marks are erased in the developing germ cell.

Surani is now researching how mitochondria—the cells' energy source—perform during the egg-making process. Mitochondria go through a selection process during reproduction, with the child receiving only his or her mother's genetic material. The process of correcting defects in mitochondria is not well understood, but Surani hopes that by studying how the germ cell corrects such errors, he and his colleagues can learn a lot about cellular energy and related diseases. "Along the way, we can gather knowledge that could have a huge impact on human health," he says.

Hayashi hopes that such efforts will also be useful for rescuing and restoring nearly extinct species, such as the white rhinoceros. By improving their understanding of the process of forming gametes, researchers will be better poised to work with species that are likely to die out, he says. He is currently trying to reproduce his mouse research in northern white rhinos. Although progress is coming slowly, Hayashi thinks artificial insemination will eventually allow scientists to rescue the nearly extinct species. But the wait time is much longer. A mouse is pregnant for 20 days; a white rhino is pregnant for 16 months, he notes.

When Hayashi talks to audiences about his white rhino work, everyone looks happy, he says. But when he mentions doing similar research in humans, "some people are very skeptical, and some are very afraid." Hayashi understands their concerns. A lot of human germ cells and embryos would be wasted before human stem cells could successfully be transformed into viable eggs and sperm. Even the viable gametes might still carry the risk of birth defects, he cautions.

Reijo Pera believes that ethics do support studying this work for human applications—and, if it can be done safely enough, even using it to create humans. A cancer survivor who is infertile herself, Reijo Pera believes that helping couples have children justifies the quest.

Yet thorny questions remain about exactly what should be considered safe and who should decide that. When scientists developed other contentious technologies, such as IVF and the gene-editing CRISPR system, formal meetings among researchers, ethicists and members of the public helped to develop recommendations and guidelines for their potential applications. The same will likely be required for in vitro gametogenesis, researchers and ethicists note. Moreover, those conversations should take place long before the science is at a stage where humans can use it. "Before the inevitable, society will be well advised to strike and maintain a vigorous public conversation on the ethical challenges of [in vitro gametogenesis]," Daley and his colleagues wrote in their January 2017 paper. "With science and medicine hurtling forward at breakneck speed, the rapid transformation of reproductive and regenerative medicine may surprise us." SA

Karen Weintraub is a freelance health and science journalist who writes regularly for the *New York Times,* STAT and *USA Today,* among others.

MORE TO EXPLORE

Derivation of Oocytes from Mouse Embryonic Stem Cells. Karin Hübner et al. in *Science,* Vol. 300, pages 1251–1256; May 23, 2003.

Offspring from Oocytes Derived from In Vitro Primordial Germ Cell–Like Cells in Mice. Katsuhiko Hayashi et al. in *Science,* Vol. 338, pages 971–975; November 16, 2012.

Reconstitution In Vitro of the Entire Cycle of the Mouse Female Germ Line. Orie Hikabe et al. in *Nature,* Vol. 539, pages 299–303; November 10, 2016.

scientificamerican.com/magazine/sa

© 2019 Scientific American

© 2019 Scientific American

VANQUISHING Diabetes

Cleaner environments in the industrial world have led to an increase in the incidence of type 1 diabetes. This history shows the way to a novel vaccine

By Kristen M. Drescher and Steven Tracy

Illustration by Matt Harrison Clough

© 2019 Scientific American

ALMOST THREE DECADES AGO A BRITISH EPIDEMIOLOGIST NAMED DAVID P. STRACHAN proposed a simple, if counterintuitive, idea to explain why hay fever, eczema and asthma had become increasingly common over the preceding century. Strachan linked rising rates of these allergic illnesses in the U.K. with improvements in living standards since the industrial revolution—in particular, a sharp drop in the number of infections experienced in early childhood. He surmised that exposure to bacteria and viruses in the first years of life (provided an infant survived them) somehow protected against these conditions showing up later.

Although Strachan's original hunch, now commonly known as the hygiene hypothesis, concerned allergic disorders, researchers have since used its basic tenet—exposure, or lack thereof, to environmental influences—to explain historical increases in various other conditions as well. These include poliomyelitis, multiple sclerosis and type 1 diabetes. Numerous epidemiological surveys have revealed certain escalating disease patterns as industrialization spread from Europe to North America and beyond. Wherever the rate of childhood infections (and mortality) fell, the incidence of several previously rare illnesses started to rise—albeit not uniformly and not all at once.

Major polio outbreaks first began to appear in the late 1800s. The incidence of multiple sclerosis, in which the immune system attacks the protective covering around particular nerve cells, doubled in certain parts of the world in the second half of the 20th century. Type 1 diabetes, which occurs when the body mistakenly destroys cells in the pancreas that make the hormone insulin (which enables the body to use glucose for energy), started creeping up in the first half of the 1900s and rose dramatically in the 1950s.

Exactly how early exposure to various viruses or bacteria can protect against the emergence of a number of seemingly unrelated illnesses remains unclear. Somehow the infections enable the developing body to learn how to deal with pathogens. Further, the absence of exposure to these microbes can prompt the body to attack itself. In particular, a substantial collection of research implicates a fairly large group of pathogens called enteroviruses in the surge of polio and type 1 diabetes.

Unlike the far more common type 2 diabetes—often tied to weight gain in adulthood—type 1 diabetes usually strikes before the age of 20. Our experiments on mice that are prone to spontaneously acquiring type 1 diabetes have revealed a complex mechanism whereby the same strains of enteroviruses can either prevent or instigate the illness, depending on the age of the mouse when it is infected. Assuming our results are confirmed in humans, a vaccine based on a group of viruses commonly excreted in feces could potentially prevent type 1 diabetes in many individuals.

A CENTURY OF CLUES

OUR RESEARCH BEGAN with a fundamental question, similar to the one Strachan had addressed: Why was type 1 diabetes so rare in the past and yet by the 1950s had become a scourge? In ancient times, Greek, Arab, Indian and Chinese physicians all described a rare cluster of symptoms—including rapid weight loss, abnormal thirst and urine that tasted sweet when sampled—that were almost certainly an outcome of type 1 diabetes. Extrapolating from individual hospital data, researchers calculate that about one or two in every 100,000 children under the age of 15 developed type 1 diabetes in the early 1900s. Today that number is closer to 20 per 100,000 children in parts of the U.S. and more than 60 per 100,000 in Finland. Disturbingly, these numbers continue to rise.

The increase has not been steady, however. After years of just creeping up in some countries, type 1 diabetes began to soar in the middle of the 20th century. Since then, epidemiologists have calculated an average annual increase of between 3 and 5 percent across the globe. Between 1998 and 2010 the incidence of type 1 diabetes jumped by a shocking 40 percent.

Such a steep rise in such a short time told us that we were not

IN BRIEF

Improved sanitation in the developed world led to historical increases in the incidence of certain diseases, including polio and type 1 diabetes.

Certain viruses commonly found in untreated sewage seem to precipitate and protect against type 1 diabetes, depending on the age at infection.

Vaccines that derive from such viruses may safeguard genetically vulnerable individuals against acquiring type 1 diabetes.

© 2019 Scientific American

looking at a fundamental change in the human gene pool: DNA does not mutate that rapidly. A variety of different combinations of many genes profoundly increase the risk of an individual acquiring type 1 diabetes. So far as investigators can tell, however, the prevalence of these high-risk genetic profiles has not changed. Instead more and more people with a relatively low genetic predisposition to type 1 diabetes are now suffering from the ailment. Few cases can be linked solely to a genetic disorder. These and other findings, from scientists around the world, strongly suggest that newly emergent environmental factors must be at work.

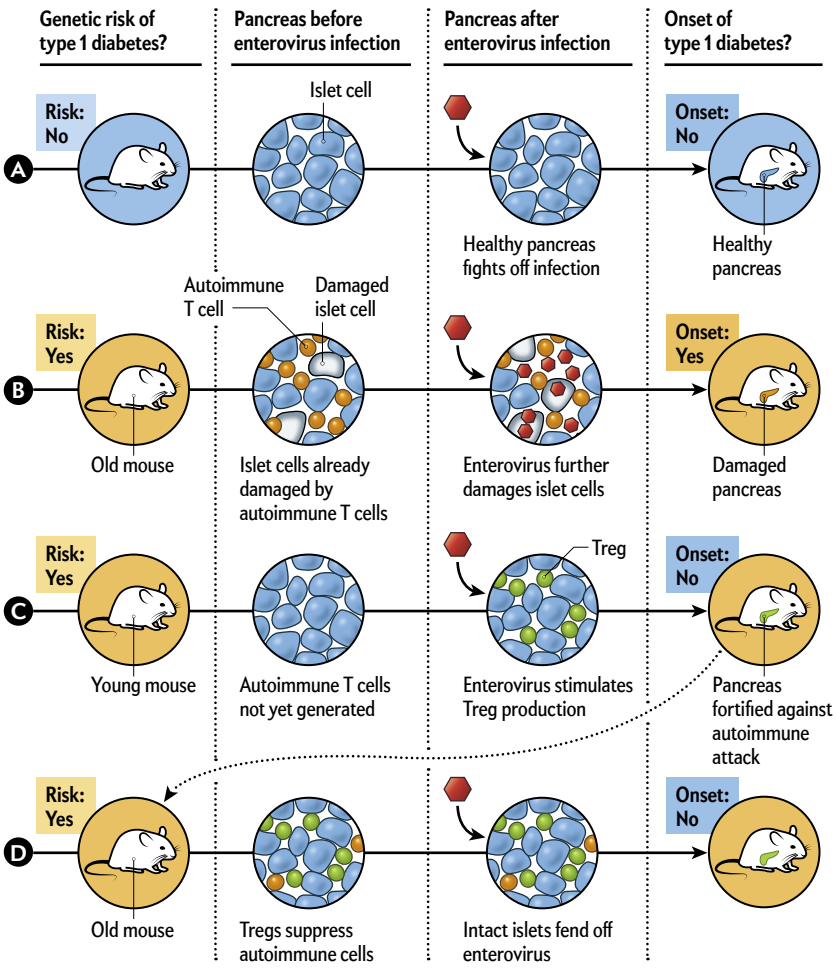
Various possibilities have been considered over the years—and dismissed. Unlike type 2 diabetes, type 1 does not originate in a person's diet. More intriguingly, several studies have determined that type 1 diabetes occurs more frequently the farther away one gets from the equator. Might a lack of vitamin D, which is easily produced by the body whenever it is in sunlight, account for such regional variation? That idea soon fell apart, however. Epidemiologists discovered, for instance, that some countries in the far north, such as Finland, had higher rates of type 1 diabetes in regions with more sunlight than in regions with less.

The bulk of the evidence points instead to a viral trigger—probably one or more viruses that occur in sewage or in contaminated drinking water. Numerous studies indicate that enteroviruses—so named because they are normally found in the intestine (*énteron* in ancient Greek)—are the culprits. (Indeed, no substantive data link any other kind of viral or environmental influences to the disease.) Some enteroviruses are able to replicate in the pancreas, inflaming areas adjacent to where the islet cells, which produce insulin, reside. The inflamed regions produce autoimmune T cells, which in certain circumstances protect the body against invaders. Autoimmune T cells, however, attack the body's own islet cells, destroying its ability to produce insulin and thereby bringing on diabetes.

Researchers have counted more than 100 types of enteroviruses. No single type of enterovirus, however, seems solely responsible for detonating diabetes around the world. Rather scientists have identified a number of candidates, chief among them six enteroviruses called the Coxsackie B viruses, implicated in the precipitation of the ailment. And they do not understand exactly how such infections might provoke the body to attack itself. The process must be complex: epidemiological studies indicate that specific enteroviruses that appear to advance the disorder in some people apparently protect others from developing it in the first place.

Enterovirus Infections and Type 1 Diabetes

A mouse that is free of genetic vulnerability to type 1 diabetes **A** readily fights off an enterovirus infection, and the islet cells in its pancreas continue to produce insulin. How a mouse with genetic predisposition to type 1 diabetes **B**, **C**, **D** responds to an enterovirus infection depends on its age. An older mouse **B** is likely to have its islet cells already damaged by spontaneously generated autoimmune T cells when the enterovirus attacks. In that case, the enterovirus reproduces in the islets, further damages them and reduces the production of insulin—triggering the onset of type 1 diabetes. If, however, the mouse is young, and an autoimmune attack is not yet underway **C**, the infection prompts the production of regulatory T cells (Tregs), which suppress the generation of autoimmune T cells. The Tregs subsequently fortify the pancreas against type 1 diabetes **D**.



Graphic by Amanda Montañez

© 2019 Scientific American

DIRTY WATER

AS WE EVALUATED the kinds of experiments that could potentially identify diabetes-causing enteroviruses, we sought clues in a different illness: polio. An often paralyzing childhood affliction, polio is caused by another class of enteroviruses, called polioviruses. These viruses seem to have been around for millennia: an ancient Egyptian stele (a relief carved in a stone slab), now in a Copenhagen museum, appears to depict a polio patient. The terrible condition they engender used to be quite rare, however, until the late 19th century, when sporadic and then annual epidemics suddenly began to occur. Polio killed tens of thousands of children and crippled millions more in the 20th century. As recently as 1988, polio crippled about 1,000 children a day. Thanks to a massive vaccination campaign, polio is now endemic in only three countries.

The hygiene hypothesis helps to explain this sudden surge in polio cases. It is easy to forget that many conveniences of contemporary life in the developed world are only about a century old. Before the widespread installation of municipal water supplies in Europe and North America, people drew water from wells, ponds or public fountains for all purposes, including drinking, bathing and washing clothes. Unsurprisingly, drinking water was often contaminated with human or animal waste. The paucity of running water and soap also meant that people could not be as strict about keeping their hands clean after going to the bathroom as they can be today. Consequently, the simple act of preparing a meal or shaking hands could spread germs far and wide.

Thus, nearly everyone, from early life onward, was exposed to polioviruses that had been passed into the environment from human feces. Newborns did not tend to develop the illness, however, because the mothers had developed immunity against the viruses and passed on protective antibodies to both the developing fetuses during pregnancy and later, while nursing, to their babies. As infants grew older and stopped nursing, and maternal antibodies waned, young children began to make their own protective antibodies against the virus because of repeated exposures to it. So although polioviruses were almost ubiquitous, polio itself was uncommon because youngsters were first protected by the mothers' antibodies and were subsequently protected by their own immune system.

This chain of protection, a part of human life for eons, began to break as human populations entered a "cleaner" world. A boy who might have been spared exposure to polioviruses could encounter them later in life, when he had no protective immunity. Such a chance encounter with poliovirus could then lead to paralytic polio—once in every 100 to 200 poliovirus infections. That is probably why President Franklin D. Roosevelt, for example, developed paralytic polio at the age of 39 while on vacation on an island in New Brunswick, Canada.

Despite polio's grim legacy, we took some consolation from the fact that a vaccine for the viral infection that causes it had proved safe and immensely effective. If researchers can make a vaccine against one type of enterovirus, they should be able to make a vaccine against others as well. And if experiments prove that enteroviruses cause type 1 diabetes, the discovery might point to a potential new treatment: namely, a vaccine against type 1 diabetes that would protect those at greatest risk from acquiring the viral infection in the first place.

(We can rule out poliovirus as a cause of type 1 diabetes. Although polio epidemics became commonplace in the 20th century, no parallel outbreaks of type 1 diabetes were observed. In addition, polio has been eradicated from countries where the incidence of type 1 diabetes continues to rise.)

To show that a virus causes a particular disease, one should begin by isolating the virus from the affected tissue. For type 1 diabetes, that is the pancreas. But safely sampling tissues from a human pancreas remains a surgical challenge—which is why such biopsies are seldom performed on people who are otherwise healthy. Furthermore, pinning down the exact moment when the body's immune system starts attacking the pancreas and destroying the insulin-producing islet cells is extremely difficult. By the time it becomes clear that someone has type 1 diabetes, any signs of what may have been an active infection have usually disappeared.

Nevertheless, about 40 published reports convincingly link the presence of various enteroviruses to the onset of type 1 diabetes: either the virus or its genetic material was isolated from patients' postmortem pancreatic tissues. And other studies have shown that some kind of enterovirus infection most likely plays a long-term role in the development of type 1 diabetes.

As it happens, a particular strain of mice, known as the nonobese diabetic (NOD) mouse, acquires type 1 diabetes on its own, without any intervention from investigators. (Curiously, NOD mice maintained in hygienic conditions acquire the disease much faster than those in dirty cages.) We hypothesized that NOD mice resemble humans with a genetic predisposition to type 1 diabetes. Furthermore, unlike most enteroviruses, the Coxsackie B viruses replicate well in mice and had already been linked to type 1 diabetes. All these factors made NOD mice the ideal model for exploring the relation between enteroviruses and type 1 diabetes.

In 2002 we deliberately infected very young NOD mice, otherwise held in sterile environments, with Coxsackie B viruses. We found that the animals were much less likely to develop type 1 diabetes as they aged, compared with uninfected control subjects. These results supported the hypothesis that early exposure to microbes offers a protective effect against developing type 1 diabetes. Intriguingly, that effect was not limited to specific types of Coxsackie B viruses, although some seemed to provide stronger protection than others. Experiments by virologist Heikki Hyöty of the University of Tampere in Finland and his colleagues have yielded similar outcomes.

We can think of three possible mechanisms by which exposure to enteroviruses when young might prevent the damaging impact of such infections later in life. First, an infection could trigger the development of protective antibodies against that specific type of enterovirus, so subsequent exposure to the same type would not result in disease. (This process mirrors the principle behind the poliovirus vaccines and many other viral vaccines we use today.) Second, because the Coxsackie B viruses are very similar, on a molecular level, to other enteroviruses, they may prompt the body to more rapidly mobilize adequate defenses, even to enteroviruses it has never encountered before. Third, an enterovirus infection may stimulate the production of regulatory immune cells called Tregs. These generally beneficial cells serve as a conscientious police force, suppressing autoimmune T cells that would otherwise harm the host.

To tease apart these diverse mechanisms, we decided to infect the mice at different ages and observe them for at least 30 weeks after the inoculation. After many years of experimenta-

© 2019 Scientific American

tion, we discovered that infecting older NOD mice with Coxsackie B viruses increased, rather than decreased, their likelihood of developing type 1 diabetes. This finding contrasted sharply with what we had observed in young NOD mice.

We concluded that the pancreas had to be already inflamed—meaning that the insulin-producing islet cells had to be under attack by the mouse's own autoimmune T cells to begin with—for an enterovirus to enter the islets and multiply, accelerating the onset of diabetes. In other words, a genetically induced autoimmune attack on the pancreas had to be underway before a Coxsackie B virus infection could hasten the onset of type 1 diabetes. The older the mouse and the worse the inflammation, the faster the illness would take hold—often resulting in full-blown diabetes in a day or two. (In contrast, older mice held in sterile environments developed diabetes weeks later.)

Studies by immunologist Matthias von Herrath of the La Jolla Institute for Allergy and Immunology in California and his colleagues indicated that enterovirus infections early in life (before an autoimmune attack is launched) can stimulate the production of regulatory T cells, which persist into adulthood. The Tregs suppress the production of autoimmune T cells and thereby protect against type 1 diabetes. But if the pancreas is already inflamed with autoimmune T cells—as would naturally happen in older NOD mice—the virus is able to replicate, damaging the insulin-producing islet cells and precipitating diabetes. In other words, enteroviruses can either protect against or trigger type 1 diabetes in NOD mice, depending on the age at which the infection occurs.

DIABETES VACCINES

ASSUMING THESE OBSERVATIONS in NOD mice reflect what occurs in humans with a genetic predisposition to type 1 diabetes, how might we exploit them to help such vulnerable individuals? Nobody wishes to return to the days of poor or no hygienic practices. But we should not have to. Based on our experience of poliovirus vaccines, we know that developing safe and effective enterovirus vaccines is feasible.

Generally, antiviral vaccines come in three versions: live but attenuated, killed and subunit. Live, attenuated vaccines were originally generated by passing the virus through cells or an animal host to weaken its ability to cause disease. Such vaccines are regarded as the most proficient at inducing immunity because the viruses replicate in the host and induce a normal immune response. But they can mutate rapidly into a pathogenic strain. Genetic engineering now enables specific areas of a virus's genome to be altered or deleted to limit the likelihood of such reversion, but the risk remains. Killed vaccines inactivate viruses so that they cannot multiply, but they still induce a certain level of immunity in the host. Because the virus does not persist in the body, however, periodic revaccination is usually required. A subunit vaccine uses one or more parts of a virus, which are known to stimulate an immune response to produce the desired type of immunity in the person vaccinated.

The bulk of the evidence indicates that no single enterovirus or even a few enteroviruses are involved in the onset of type 1 diabetes throughout the world. Furthermore, the historical evidence indicates that type 1 diabetes was rare when exposure to numerous enteroviruses was a fact of life. We hypothesize, therefore, that vaccinating with multiple types of enteroviruses should offer the most protection. Such an approach would simulate the way humans used to accumulate protective immunity to enteroviruses throughout history. The initial vaccine could be a killed-virus one for safely inducing immunity. Thereafter, either inactivated or highly attenuated vaccines could be used as booster doses.

We find it encouraging that a vaccine approach against type 1 diabetes is finally underway. Hyöty's group is working with Finnish biopharma Vactech Oy, for which Hyöty is chairman of the board, to develop a vaccine against a single type of Coxsackie B virus and has tested its ability to prevent type 1 diabetes in mice. This killed-virus vaccine is in a pre-clinical development stage and is yet to be tested for safety in humans. Testing it in children—to ensure safety, generation of a protective immune response to the enterovirus and protection from type 1 diabetes—will take upward of a decade. Given that numerous observations suggest that no single strain of enterovirus is involved with diabetes, we can only remain hopeful that this vaccine will significantly lower the incidence of type 1 diabetes.

In addition, a remarkable variety of efforts are in the works to reverse type 1 diabetes after its onset. Investigator Paolo Fiorina of Boston Children's Hospital and his colleagues have demonstrated that appropriately manipulated stem cells, when infused into mice, can sometimes reverse type 1 diabetes. Another group, led by Denise Faustman of the Massachusetts General Hospital Immunobiology Laboratory, is investigating the efficacy of the bacillus Calmette-Guérin (BCG) vaccine, normally used to prevent tuberculosis, in undoing the effects of type 1 diabetes. Several research groups in the U.S. and the U.K. have focused on immunization with proinsulin (a precursor of insulin) or the DNA that encodes for it. In particular, a 2017 report from a multi-investigator effort by Mark Peakman of King's College London and his colleagues indicates that a protein fragment from proinsulin can induce beneficial responses in people newly diagnosed with type 1 diabetes.

Doctors in the U.S. alone diagnose 40,000 new cases of type 1 diabetes every year. We need to remember that a vaccine cannot entirely eradicate the disease: some cases seem to occur solely because of the patient's genetic makeup. Refining treatments to improve the quality of life for individuals who can no longer make their own insulin remains important. Even if only a small fraction of participants in vaccine trials are protected from the disease, however, a significant number of people will have better lives. Given how fast the incidence of type 1 diabetes is increasing, the ability to make it as rare as it once was could benefit millions. SA

Kristen M. Drescher is a professor of medical microbiology and immunology at Creighton University. She studies the role of viruses in autoimmune disease and explores novel therapeutics in treating inflammatory ailments.

Steven Tracy is professor emeritus of pathology and microbiology at the University of Nebraska Medical Center. His research focused on the molecular biology of enteroviruses, as well as on the impact of such pathogens on myocarditis and type 1 diabetes.

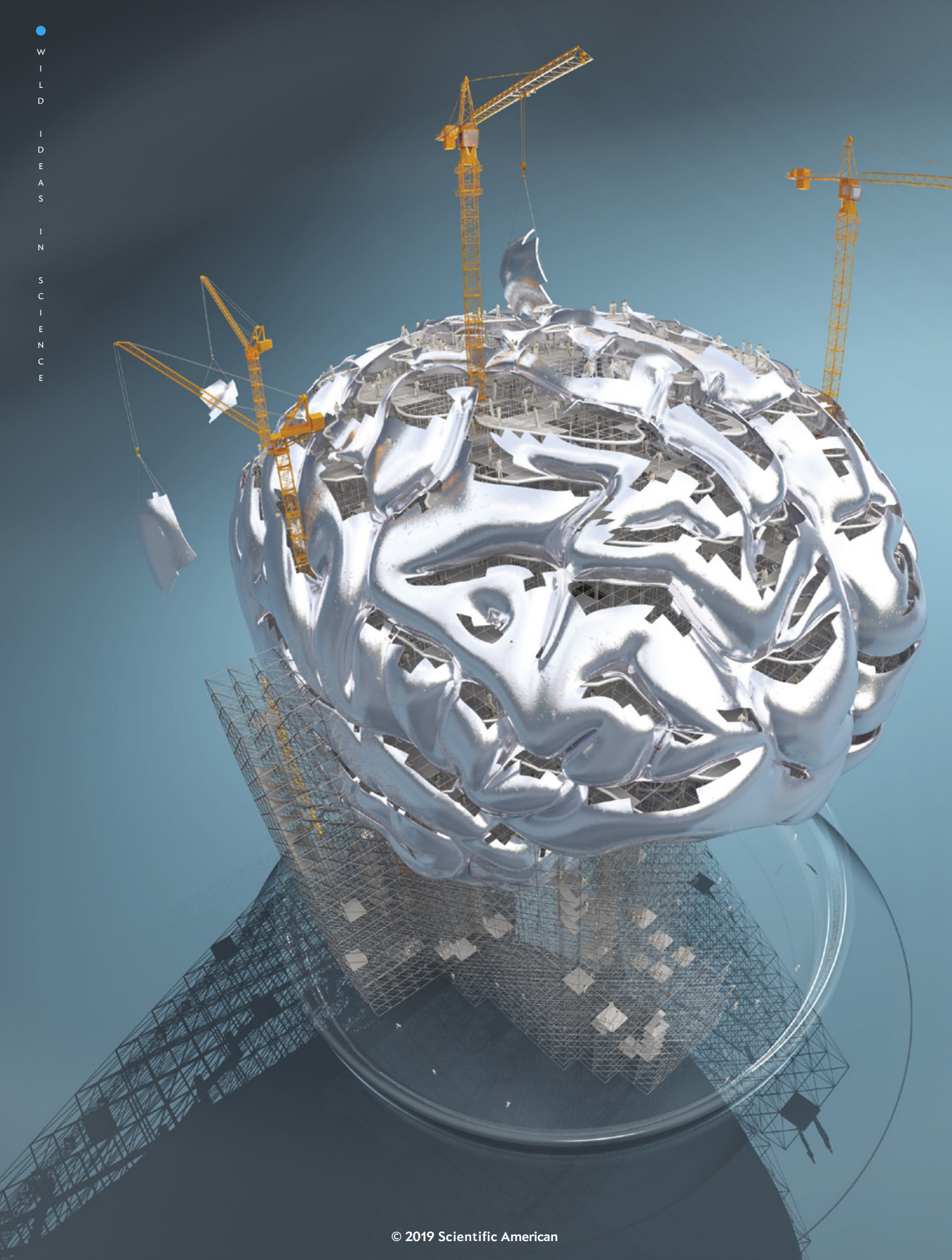
MORE TO EXPLORE

Enteroviruses, Hygiene and Type 1 Diabetes: Toward a Preventive Vaccine. Kristen M. Drescher, Matthias von Herrath and Steven Tracy in *Reviews in Medical Virology,* Vol. 25, No. 1, pages 19–32; January 2015.

Developing a Vaccine for Type 1 Diabetes by Targeting Coxsackievirus B. Heikki Hyöty, Francisco Leon and Mikael Knip in *Expert Review of Vaccines,* Vol. 17, No. 12, pages 1071–1083; 2018.

scientificamerican.com/magazine/sa

© 2019 Scientific American



© 2019 Scientific American

"What I cannot create, I do not understand."
—Richard Feynman, 1988

LAB-BUILT BRAINS

Scientists copy nature's most complex organ in the hope of solving the mysteries of brain disorders, from autism to Alzheimer's

By Juergen A. Knoblich

Illustration by Bryan Christie

© 2019 Scientific American

EVERYTHING THAT MAKES US HUMAN IS LOCATED WITHIN 1.4 KILOGRAMS of yellowish tissue composing the human brain. It is here that our thoughts develop, here that we feel love or hate, and where the most creative and most evil ideas of humankind arise. This walnut-shaped structure is also the most complex organ nature has generated. The brain harbors about 86 billion neurons, or nerve cells, that have to be born at the right time, migrate to the right place, and wire up in the right way if we are to survive and thrive.

Understanding exactly how the human brain develops and functions is the greatest challenge of modern biology. Most of what we have learned about the organ since the birth of neuroscience more than 100 years ago derives from experiments done on animals—frequently mice or rats. Scientists could justify this approach because mice and humans share a common brain architecture: they have many of the same types of nerve cells and rely on essentially the same parts of the brain to carry out shared mental processes. But humans and rodents differ in one key way. Whereas the mouse brain has a smooth surface, the human brain is highly folded.

To nonscientists, this difference might seem trivial. But neurobiologists believe that the folding makes a world of difference to human brain function. It allows for many more neurons to be placed within the same volume and is also a prominent feature of all "intelligent" animals, such as monkeys, cats, dogs and whales. Evolutionary biologists have shown that folding arose from another difference between mice and people: neurons in many parts of the brain arise from a specific set of precursor cells that exist only in minute numbers in mice.

Such differences may explain why many common genetic mutations responsible for severe neurological disorders in humans have little effect when bred into mice by researchers trying to study the mechanisms of human diseases. If the mutations affect the development or maintenance of proper human brain architecture or the functioning of cell types that are common only in humans, then the studies would be doomed to failure. In fact, the unique characteristics of the human brain may be one of the reasons that rodent studies have yielded no effective therapies for such brain disorders as schizophrenia, epilepsy and autism.

Recognition of the differences between mouse and human brains has spurred a hunt for more informative ways to conduct neuroscience experiments. Recently my laboratory has come up with an exciting approach: growing the largest part of the developing brain in miniature in a lab dish. These brain structures, called organoids, give neuroscientists a model of the human brain that should provide information they cannot obtain by running studies in mice. Researchers can observe what happens when the brain-in-a-dish, or mini brain, is exposed, for example, to the Zika virus, which can disrupt brain development in fetuses of infected women, or when an organoid is genetically engineered to mimic a brain afflicted with a neurological disease.

BRAIN-IN-A-DISH (SORT OF)

MY LAB BEGAN WORK on organoids in 2012, when Madeline A. Lancaster, then a postdoctoral scientist in the group, devised a way to replicate in a culture dish the essential processes that lead to brain formation in a human fetus during the first roughly 10 weeks of development [*see box on opposite page*]. Our procedure relies on human cells known as stem cells, which exhibit a remarkable feature called pluripotency. Pluripotent stem cells are the same type of cells found in the early embryo. When cultured under the right conditions, they can give rise to any kind of tissue, be it nerve, muscle, blood, bone or any other type. In the fetus, these new cells retain their pluripotency for only a few days. But using special lab cultures, researchers can preserve them in this state permanently and ultimately turn them into almost any desired cell type.

To start, we culture the cells in a liquid containing all the nutrients needed for growing the neuroectoderm, the part of a fetus that forms the nervous system. When the cells aggregate into a ball called an embryoid body, we embed the ball in an amazing substance called Matrigel. This gel, produced by cultured cells that were isolated from a mouse cartilage tumor,

IN BRIEF

Knowledge about the human brain often derives from experiments performed on mice, rats or other animals. Brains of these species share much in common with the human organ, but they lack a highly folded surface, a difference that affects neural functioning.

Unique qualities of the human brain may help explain why rodent studies have failed to yield new treatments for brain disorders ranging from schizophrenia to Alzheimer's disease. That has spurred a search for new ways to conduct neuroscience experiments.

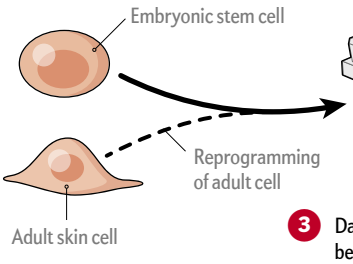
One alternative entails growing the largest part of the developing brain in a laboratory dish. These "organoids" most likely will give brain scientists information that cannot be obtained from mouse studies; they are already being used in investigations of the Zika virus.

© 2019 Scientific American

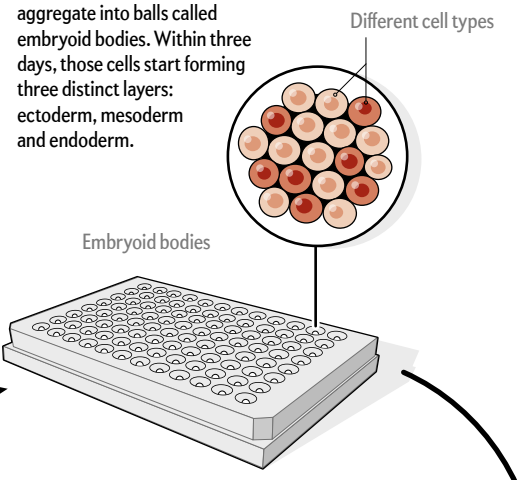
Grow Your Own

The technology that coaxes stem cells to develop into different types of biological tissue has now been used to grow a part of the brain that contains the cortex and other structures and is responsible for such higher mental functions as processing information from the outside world, forming memories and making decisions. To create such a mini brain, researchers give a tiny ball of cells nutrients and a bed on which to grow; then the cells recapitulate much of the developmental process that occurs in the early embryo.

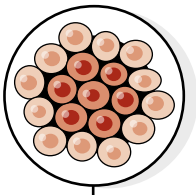
1 The procedure begins with embryonic stem cells or induced pluripotent stem cells capable of turning into any cell type in the body. The latter cells can be derived from adult skin or blood cells that have been genetically altered.



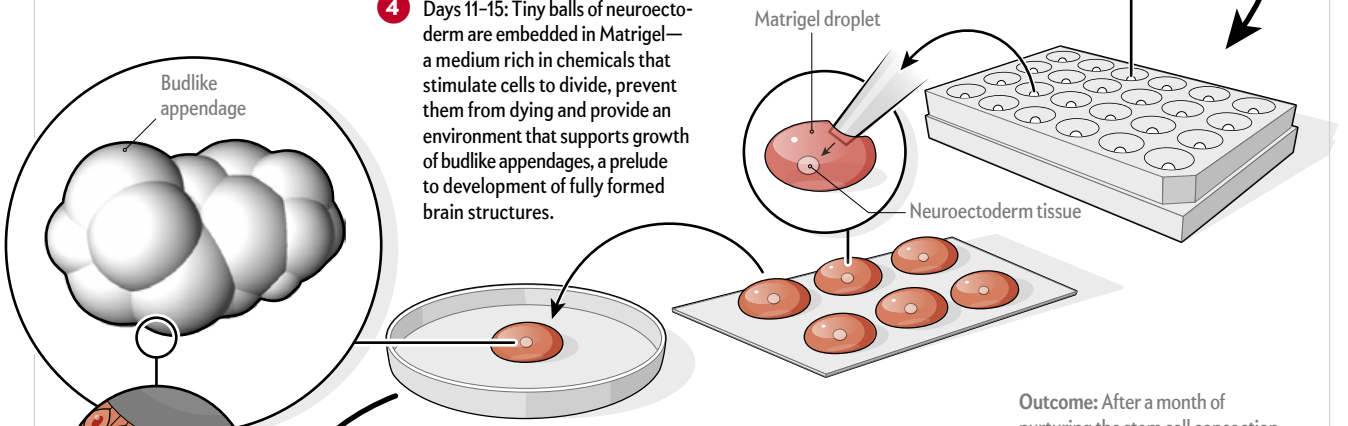
2 Days 0–5: The cells divide and aggregate into balls called embryoid bodies. Within three days, those cells start forming three distinct layers: ectoderm, mesoderm and endoderm.



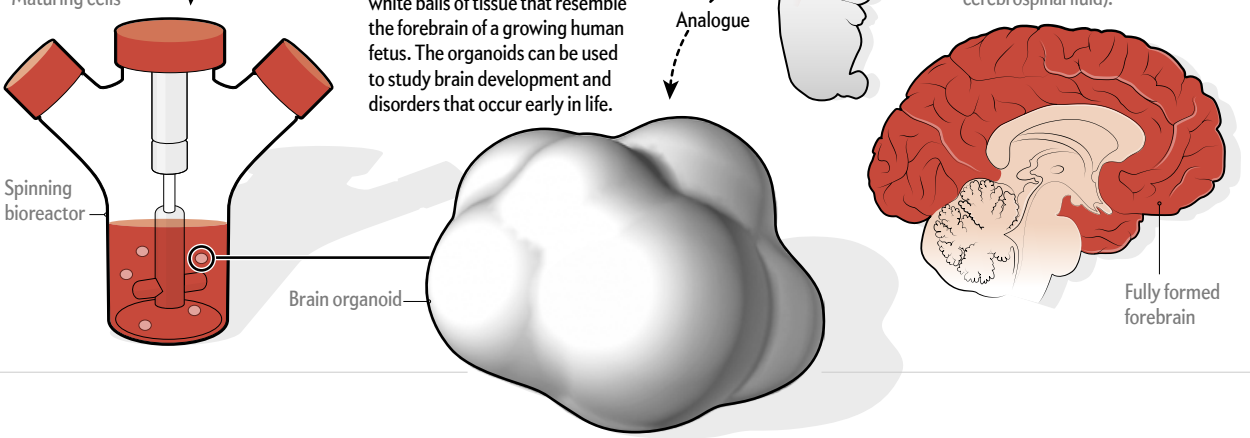
3 Days 6–10: Embryoid bodies, after being placed in a liquid containing the nutrients for the part of the fetus that forms the nervous system (the neuroectoderm), begin to cluster into layers that form the embryonic tissues that give rise to the human brain.



4 Days 11–15: Tiny balls of neuroectoderm are embedded in Matrigel—a medium rich in chemicals that stimulate cells to divide, prevent them from dying and provide an environment that supports growth of budlike appendages, a prelude to development of fully formed brain structures.



5 Days 15–30: Matrigel droplets are transferred to a spinning bioreactor or a device known as an orbital shaker. In the gel, the embryoid bodies grow into brain organoids—three-dimensional, white balls of tissue that resemble the forebrain of a growing human fetus. The organoids can be used to study brain development and disorders that occur early in life.



Outcome: After a month of nurturing the stem cell concoction, the cultures are strikingly similar to the forebrain of a 10-week-old embryo. This brain region includes the cortex (the large, folded outer structure) and the choroid plexus (the region that generates cerebrospinal fluid).

SOURCE: "GENERATION OF CEREBRAL ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS," BY MADELINE A. LANCASTER AND JUERGEN A. KNOBLICH, IN *NATURE PROTOCOLS*, VOL. 9; OCTOBER 2014

Illustration by Tami Tolpa

© 2019 Scientific American

resembles the membrane on which cells sit in the fetus. Matrigel, which is rich in factors that both stimulate cells to divide and prevent them from dying, provides a scaffold that is stiff enough for cells to grasp but malleable enough to be modified by the cells, which in turn alter its shape.

The outcome of these experiments has been truly spectacular. Left to their own devices in the gel, the embryoid bodies grow into three-dimensional, white balls of tissue that resemble the embryonic human brain. Exposed to the proper chemical signals that trigger fetal brain development, stem cells grow into exact replicas of the human forebrain, the region responsible for higher mental functions. It includes such components as the cortex (the large, folded outer structure) and the choroid plexus (the region that generates cerebrospinal fluid). We also find other structures that guide cells to their proper place in the developing brain. The medial and lateral ganglionic eminences, which perform this function, assist in giving rise to cells that generally tamp down neural activity (interneurons) and the hippocampus, which is involved in memory formation.

Cells in a growing organoid arrange themselves identically to those in the brain of an eight- to 10-week-old human fetus. In rare cases, the organoids even grow small eyecups, indentations in the tissue that contain colored pigments, much as occurs when the human eye begins to form. Also, as happens in a developing brain, the cells divide and give rise to the kinds of nerve cells found in an embryo. And the nerve cells send out axons—long cables that make contact with other neurons to form an active signaling network. Before forming these networks, the neurons migrate from one area to another, much in the way they do in the fetus, potentially providing clues to what happens when neurons end up in the wrong place, as they often do in psychiatric disorders.

WE HAVE DEMONSTRATED TECHNICAL FEATS WE COULD ONLY DREAM OF A FEW YEARS AGO.

ON THE SHOULDERS OF GIANTS

THE IDEA OF BUILDING TISSUES in culture is not really new. As with most scientific discoveries, the current organoid boom relies on years of pathfinding research, some of it dating back more than a century. Already in 1907 zoologist Henry Wilson had demonstrated that certain lower animals, such as sponges, can put themselves back together after being broken up into single cells, an indication that the brain is endowed with a program for assembling its myriad parts.

In 1939 Johannes Holtfreter discovered that the various cells in a frog embryo will seek one another out and regenerate their shape even after they have been completely separated. During the 1980s this finding led to a huge boom in "reaggregation" studies, in which complex animal organs such as the retina and even the cortex were formed in the lab by bringing together their diverse cell types.

Building on early reaggregation experiments conducted from 2006 to 2010, the late Japanese scientist Yoshiki Sasai of the RIKEN Center for Developmental Biology pioneered the use of pluripotent stem cells for growing nervous system tissue, most notably the human retina. In fact, our brain organoid technology merged his techniques with groundbreaking work by Hans Clevers of Utrecht University in the Netherlands, who combined stem cells with Matrigel to establish a culture system that can be used for growing gut, stomach, and even liver and pancreatic tissue.

Beyond drawing lessons from these earlier studies, our work makes use of recently developed technologies that are dramatically turning the entire field of biomedical research upside down. One called reprogramming was developed by Japanese Nobel Prize laureate Shinya Yamanaka of Kyoto University. Through a simple set of genetic manipulations, reprogramming turns body cells that have already fully matured back into pluripotent stem cells—and it can do so for virtually any cell, from skin to blood cells. Stem cells from a sample of skin or blood can then be transformed into various types of brain cells, and those cells can then be grown into organoids. The approach can thereby avoid the need to use cells derived from embryos.

Reprogramming allows an organoid grown from the cells of a patient with a genetic disorder to be compared with ones from a healthy individual to ferret out underlying causes of a disease, because the genetic defect in the patient's cells should afflict the organoid much as it affects the developing fetus. In fact, we have already used the organoid technology to gain insight into microcephaly, in which patients are born with a brain of severely reduced size. We found that organoids grown from cells of a patient with microcephaly are much smaller than normal. Because we can grow the patient's cells in unlimited numbers, we can now undertake detailed analyses of the chain of molecular events that leads to microcephaly in a developing fetus. Much the same should be true for other brain disorders: using patients' cells to grow organoids may enable neuroscientists to better understand the defects in brain formation that underlie schizophrenia, epilepsy and other diseases that are difficult or impossible to study in animals.

Organoids derived from the reprogrammed cells of individuals who are not ill can also be useful. Indeed, they were put to good use during the Zika epidemic, which has been blamed for causing microcephaly in a number of babies born to women infected during pregnancy. Multiple labs working on organoids, first in Brazil and then in the U.S., have now established that the virus can lead to microcephaly—a link that would have remained hypothetical were it not for this new technology. When organoids are infected with the Zika virus, their nerve cells die and the resulting organoids are much smaller than their uninfected counterparts, much like the ones we have grown from our microcephaly patient.

Organoids most likely will help with research on other viruses as well. Multiple viruses, such as cytomegalovirus or herpes simplex virus, cause brain defects when infections occur during pregnancy. By growing organoids and infecting them with different viruses, we can try to understand why they cause damage, what they have in common and how the damage mechanisms differ from one another. Ultimately organoids may be used to identify the docking points, or receptors, used by the viruses to gain entry to cells—and they may be critical for test-

© 2019 Scientific American

ing potential antiviral drugs before moving them into clinical trials with patients.

A second technique propelling the use of organoids is genome engineering—a collection of methods that allows researchers to alter a cell's genetic code. Organoids engineered to incorporate mutations suspected of causing disease can enable researchers to determine whether the genetic defects actually do lead to illness. Investigators may someday be able to evaluate whether repairing those mutations would generate healthy organoids; if so, the work could lead to new treatments that counteract the mutations' effects.

Neuroscientists are eager to explore still other applications of mini-brain technology, such as drug development. The technology can assess whether new medications affect brain tissue in desired ways, obviating the need for animal testing and thus saving on the costs of drug development. The organoids can also let scientists identify unwanted effects on the developing human brain, thereby preventing drugs that would be harmful during gestation from ever reaching a pregnant woman. If the notorious drug thalidomide, which disrupts the developing brain early in pregnancy and causes other birth defects, had been tested in this way, it presumably would not have been prescribed for morning sickness in the late 1950s and 1960s.

Organoids are becoming an invaluable tool for evolutionary biologists. They can be used to identify genes responsible for the enormous size of the human brain compared with other primates. Contrasting human and primate genomes has already identified genes that might be responsible for cognitive functions, such as language, that are unique to humans. Understanding the workings of these genes has remained largely a matter of speculation. Now researchers have already generated organoids from chimpanzees and macaques and compared them with their human counterparts to identify key differences. Organoid technology should eventually allow us to test the role of those differences by replacing human genes with their monkey counterparts and studying the effects on organoids.

SHOULD WE BE AFRAID?

THE IDEA OF GROWING a human brain in a dish is sure to make some people squeamish. Movies such as *The Matrix* come to mind that evoke fantasies about lab-grown brains developing thoughts or even personalities. These are needless fears. The probability that a lab-grown brain will develop a mind of its own is nil. An organoid is not a "humanoid" in a jar and will not be one even in the far future. Any conscious being needs to be able to process information from the senses to develop an internal mental model of reality. Organoids are neither able to see nor hear and lack any sensory input. Even if we were to connect them to a camera and a microphone, the incoming visual and auditory information would still need to be translated into a form that could be understood by these brain cells in a dish—and, as things stand, providing that translation is an insurmountable technical challenge.

Organoids are not functional brains, only lumps of tissue that imitate the molecular and cellular functioning of the organ at spectacular levels of detail. They are similar to pieces of tissue removed during brain surgery, not conscious beings.

Still, growing an organoid does raise certain ethical and legal issues. All organoids derive from cells taken from individuals who have certain legal rights. As such, performing this work in the lab must conform to the same set of legal and ethical procedures used for samples taken from patients in any industrial country. Patients, of course, must give permission before their cells can be used for research. The same set of rules applies with organoids. But even when the benefits are clearly explained, donors may not at first feel comfortable with the idea of having their cells cultured into brainlike structures.

WHAT NEXT?

THE BENEFITS of this cellular technology outweigh any possible downside. Cerebral organoids have laid the foundation for performing realistic medical and toxicology experiments in human tissue, without the need for animal experiments. Even so, I and others would like to improve them. For instance, the current generation lacks blood vessels. That absence is not a problem during the early stages of organoid development, but over time cells start dying from lack of oxygen and nutrients. In theory, it should be possible to provide blood vessels, either through new 3-D-printing techniques or by growing them from stem cells. Blood vessels are known to grow into the brain, a process that could potentially be recapitulated with a 3-D culture.

In another challenge, we want to make organoids that, in common with an actual brain, have front-to-back, top-to-bottom and left-to-right axes. Unlike a real embryo that has clearly defined body axes, organoids lack a front-to-back and head-to-tail axis. As a result, they develop randomly, so that their individual parts have different orientations. In the developing brain, complex signaling systems give a brain its sense of up versus down—and these same chemicals may ultimately do so for organoids as well. Modern biotechnology methods can generate tissue cultures in which the chemicals needed to spur cell growth during development are present. These techniques may eventually result in the formation of organoids with a forebrain on one end and the hindbrain at the opposite end.

We have already begun to push forward to begin to look for ways to overcome these barriers. We have demonstrated technical feats that we could only dream of a few years ago. Organoids are already helping to achieve a better understanding of disease and are assisting in developing drug candidates. The ability to grow parts of a brain and work with the living sample has begun to open an entirely new chapter in biological research by providing vastly more realistic lab cultures—and at times even a reasonable alternative to using animals in doing research. SA

Juergen A. Knoblich is a senior scientist and scientific director of the Institute of Molecular Biotechnology of the Austrian Academy of Sciences in Vienna. He studies neural stem cells and the development of the fruit fly nervous system.

MORE TO EXPLORE

Organogenesis in a Dish: Modeling Development and Disease Using Organoid Technologies. Madeline A. Lancaster and Juergen A. Knoblich in *Science,* Vol. 345, page 283; July 18, 2014.

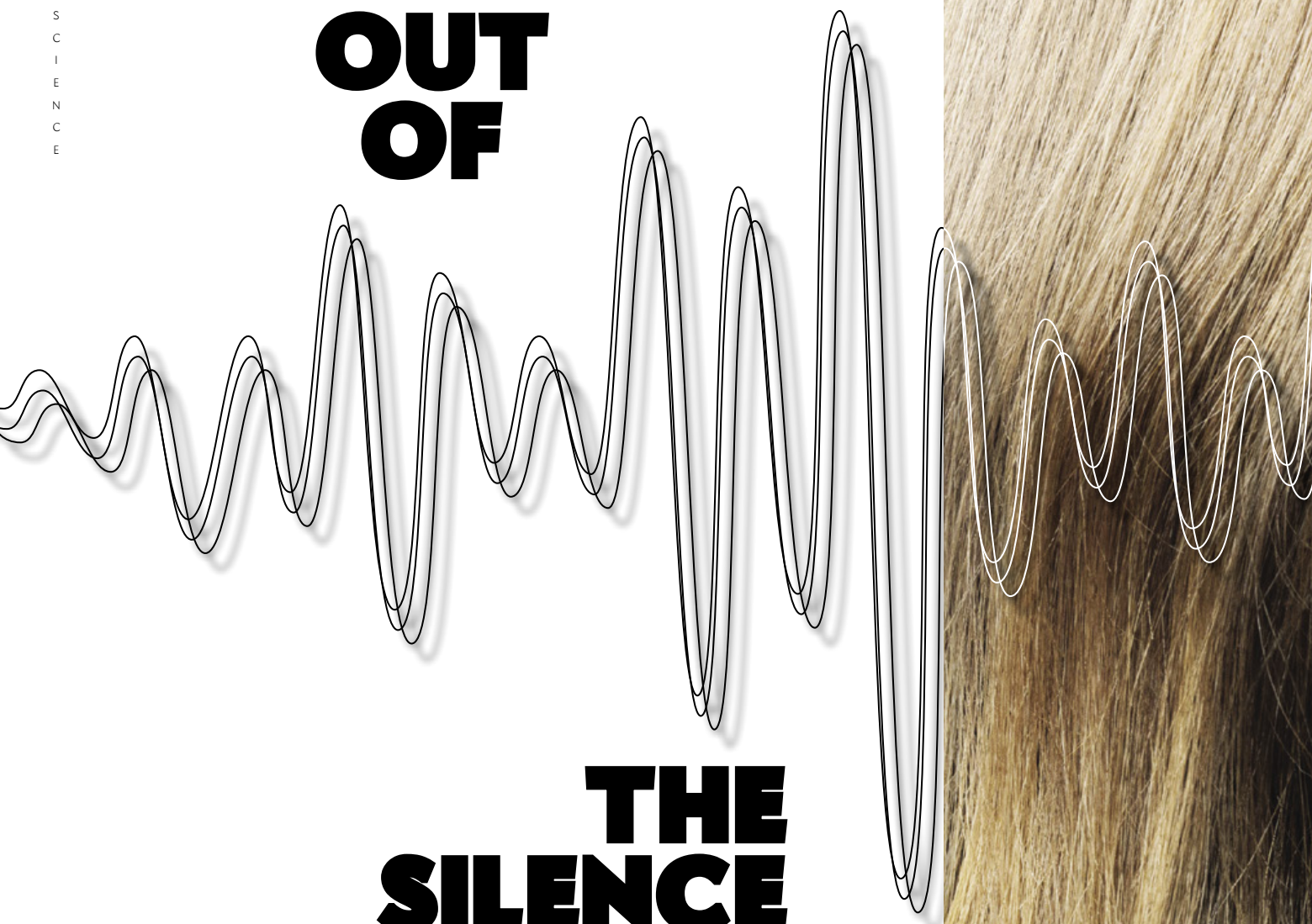
Generation of Cerebral Organoids from Human Pluripotent Stem Cells. Madeline A. Lancaster and Juergen A. Knoblich in *Nature Protocols,* Vol. 9, pages 2329–2340; October 2014.

Dishing Out Mini-Brains: Current Progress and Future Prospects in Brain Organoid Research. Iva Kelava and Madeline A. Lancaster in *Developmental Biology.* Published online July 9, 2016.

scientificamerican.com/magazine/sa

© 2019 Scientific American

OUT OF THE SILENCE



After false starts, researchers are making progress toward treating deafness with gene therapy

By Dina Fine Maron

Photographs by Ethan Hill

© 2019 Scientific American

© 2019 Scientific American

HANNAH CORDERMAN IS TRYING TO FILL IN the blanks in her world, but the blanks are growing bigger. She cannot always hear conversations, so she nods or smiles at what seem like appropriate moments, taking her cues from people around her. Picking out individual words can be hard even though doctors recently turned up the volume on her hearing aids. "There's a lot missing from conversations—bits and pieces," she says. "But I make it work."

Making it work is becoming more difficult. Corderman has an inherited condition called Usher syndrome that is slowly but inexorably robbing her of two major senses. A genetic mutation is starving cells in both her inner ear and her retina of proteins needed to detect sound and light. On top of her hearing loss, her declining vision, which forced her to give up driving at night as a teenager, has recently worsened. Now, at the age of 24, she has blind spots that make it harder to see during the day, too. No current treatment can halt or slow the disease, so Corderman lives with the knowledge that in 10 years, maybe 20 if the deterioration is slow, she could be deaf and blind.

As a child, Corderman's trouble seemed to be a straightforward hearing deficit. Then one summer in high school, as she looked up at the sky from her home in Needham, Mass., the stars seemed to blink out one by one. She told herself they were probably just covered by clouds. But the problems continued, and eventually doctors diagnosed her with Usher type 2A, a deaf-blind disorder that sets in gradually over many years. She took in the news quietly, she told me when I spoke with her. After absorbing it, she said, she decided to get on with her life. Today, seven years after her diagnosis, Corderman has finished college and works on the marketing side of her family's construction company, pushing back as much as she

IN BRIEF

Hearing loss is one of the most common birth defects, and genetic flaws are frequently the cause. Gene therapy, an experimental treatment with a checkered past, could be an important remedy.

Safety and effectiveness of the therapy have recently improved, and its use in people has been approved for several diseases. The advances in the treatment are now extending to deafness.

In a promising result, researchers were able to correct ear defects in deaf mice by injecting them with a gene-carrying virus, restoring the animals' hearing to unprecedented levels.

© 2019 Scientific American

can against a disease that she says will not define her.

There is not much doctors can do to help. Corderman could one day get a cochlear implant, a device that bypasses the hair cells to directly stimulate the auditory nerve and give her some sense of sound. But the device is a relatively blunt fix. For eyes, retinal implants—electrical stimulators for light-detecting cells—are rarely used because they do not come close to actual vision.

Corderman is not a rigorous reader of science journals, but she is aware of several hundred young mice in a few laboratories in Boston, not far from her house. The mice were bred to have auditory disorders like her own—but these mice are getting better. Using an approach called gene therapy, biologists have been dosing them with healthy DNA to make up for bad stretches that produce faulty proteins. In 2017 biologist Gwenaëlle Géléoc of Boston Children's Hospital and her colleagues reported an "unprecedented recovery" in such mice after the scientists delivered a DNA infusion to the inner ear, restoring the animals' hearing to near-normal levels. Around the same time, a separate team at Harvard Medical School reported more modest hearing restoration when they used a similar genetic approach in mice with a different hereditary disorder. A third Boston-area group recently employed gene-editing methods to knock out a faulty gene in "Beethoven mice"—rodents with another form of progressive hearing decline named after the deaf composer. The advances are providing hope that for the first time, genetic hearing disorders—some of the most common birth defects in the U.S.—could be treated and even stopped at their source.

BORN WITH Usher syndrome, Hannah Corderman has a genetic disease that is gradually stealing her hearing and her sight.

GOING VIRAL

GENE THERAPY has had a difficult and checkered past. In an infamous 1999 incident, an 18-year-old liver disease patient named Jesse Gelsinger died in an early gene therapy trial conducted by researchers at the University of Pennsylvania. A virus was used to ferry genes into his body, but the dose and type caused Gelsinger's immune system to whip itself into a fatal frenzy, attacking his own organs. The tragedy chilled public enthusiasm and funding and made many scientists cautious about going further.

Others, however, continued to quietly work with this technique, at first focusing on cells and animals, in hopes of developing therapies for complex disorders such as osteoarthritis, cancer and type 1 diabetes. As a safeguard, they lowered the doses of viruses used as delivery vehicles to keep the immune system from overreacting. They also moved away from the virus that had been used with Gelsinger, a member of a group called adenoviruses, and instead experimented with other types. One promising alternative seemed to perform particularly well in these tests: adeno-associated virus (AAV), a gene courier that appears benign to the human immune system because it does not harm our cells. Sometimes the gene-carrying viruses have been prepackaged within cells when injected, a technique that keeps them in a small target area. The field has become about "matching the right vehicle to the right disease and target and understanding dosing and where the viruses are going in the body," says researcher Cynthia E. Dunbar of the National Institutes of Health, who recently served as president of the American Society of Gene & Cell Therapy.

The improvements have worked. The U.S. Food and Drug Administration has now issued the first wave of approvals of gene therapy for people, indicating that it may be an idea whose time has finally come. In August 2017 the FDA gave a green light to Kymriah, a virus-delivered treatment for a form of childhood leukemia. And in December of that year, the agency approved the first gene therapy for a rare inherited form of blindness. Drug companies and

© 2019 Scientific American

How to Save Hearing

Some of the most common reasons for early childhood hearing loss are genetic. Inner ear hair cells are responsible for detecting sound signals and sending them to the brain, but gene mutations within those cells lead to poorly functioning anatomy. Researchers working with mice have devised a method for replacing those flawed genes with healthy versions: they put the healthy genes into a modified adeno-associated virus (AAV) and inject it into the inner ear. When the virus infects the ear hair cells, it delivers the healthy genes. If the approach (*shown here*) works in people, it could treat a major birth defect at its genetic source.

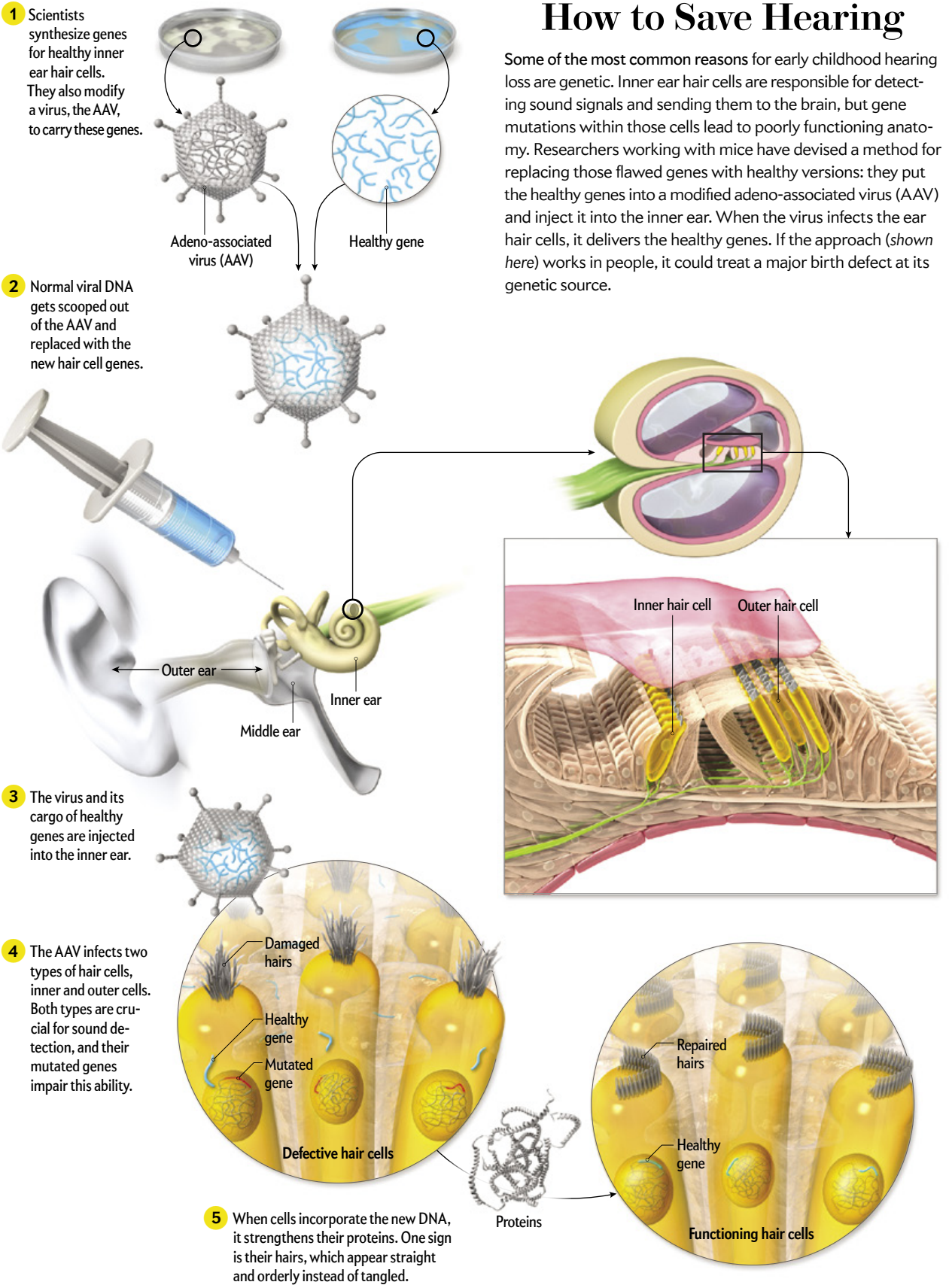

1 Scientists synthesize genes for healthy inner ear hair cells. They also modify a virus, the AAV, to carry these genes.
Adeno-associated virus (AAV)
Healthy gene
2 Normal viral DNA gets scooped out of the AAV and replaced with the new hair cell genes.
Outer ear
Middle ear
Inner ear
Inner hair cell
Outer hair cell
3 The virus and its cargo of healthy genes are injected into the inner ear.
4 The AAV infects two types of hair cells, inner and outer cells. Both types are crucial for sound detection, and their mutated genes impair this ability.
Damaged hairs
Healthy gene
Mutated gene
Defective hair cells
Proteins
Repaired hairs
Healthy gene
Functioning hair cells
5 When cells incorporate the new DNA, it strengthens their proteins. One sign is their hairs, which appear straight and orderly instead of tangled.

Illustration by Emily Cooper

© 2019 Scientific American

venture capitalists are now pouring a lot of resources into gene therapy, Dunbar says. At the society's 2018 annual meeting, about 3,400 people showed up. Only 1,200 were in attendance five years earlier.

This enthusiasm now extends to hearing loss, a disorder that is often genetic in nature. Though popularly associated with aging or accidents, hearing loss is one of the most common birth defects afflicting people; it affects as many as three out of every 1,000 babies. Genetic flaws are responsible for more than half those cases, including diseases such as Usher syndrome. Usher is a particularly appealing target because each patient has mutations in a single gene, and fixing them could fix the symptoms. Certain forms of the disease, such as Corderman's, progress gradually, which provides a window after diagnosis when treatment could stop the genetically instigated damage. That damage occurs in hair cells within the inner ear—the hairs pick up sound waves from outside and transmit them to the brain. Corderman and others like her have defective genes that grow weakened hair cells. Those cells are "like a spark plug in a car" says David Corey, one of the Harvard scientists who restored sound sensation to some mice. "Without them, hearing just doesn't work."

Gene therapy for these cells is a way to treat the root cause of the disease rather than putting on a high-tech bandage such as a hearing aid. The recent "successes are very impressive and important early promising steps," says Theodore Friedmann, a pediatrician who helps to lead a consortium of gene therapy researchers at the University of California, San Diego, and was not involved in the work. Mice are not people, of course, and the technique has yet to be tried on humans. But Friedmann says such interventions do, for the first time ever, open the door to treating deafness at the genetic level.

HAIR TREATMENT

ONE MORNING IN 2017 in a lab at Harvard, I watched Bence György lean through that door. He was actually bending over a rodent bred to have faulty hair cell genes. The mouse had been anesthetized, and György, a postdoctoral fellow who works with Corey, made a tiny incision behind the animal's ear. He pushed past paper-thin pieces of tissue as he searched for a spot in the middle ear called the round window membrane, the portal to the inner ear. When György found the membrane, he inserted a fine needle and started to slowly inject a pale pink solution containing about 200 billion particles of an AAV, each carrying a corrected form of the gene responsible for the mouse's hearing loss.

Using a virus such as an AAV to ferry that precious cargo boosts the gene's chances of arriving at its destination in the inner ear because even harmless viruses are good at infecting cells. Researchers have learned that they cannot use just any AAV, however. The hallmark of a successful AAV is the ability to infect not just one but two types of hair cells. Various AAVs are reasonably good at delivering DNA into the innermost rows of hair cells, which communicate with nerve cells, but those same viruses do a poor job of getting into the outer rows of such cells, and those are the ones that amplify sound in the first place. For full restoration of sensitivity, the vehicle needs to get into both types, says Corey, who did some of the crucial research on hair cell function.

Through a mix of trial and error and some viral gene redesign, scientists hit on a few AAVs that are drawn strongly to the two inner ear targets. They altered proteins that make up the external shell of the virus, molecules that help it bind to a cellular target. Eventually this work yielded a set of shell proteins that appear to match up with molecules on both types of hair cells, allowing the virus to make its way inside. In the paper by Géléoc and her colleagues published in 2017, researchers reported that one of these specially modified AAVs arrived intact in rodents genetically destined to be deaf at birth and produced rows of strong, well-functioning hair cells. Other research groups reported they were able to get related AAVs into the inner ear hair cells of older rodents with ears that more closely resemble those of young children.

ONE MAIN TEST OF RESTORED EAR FUNCTION INVOLVES EXPOSING THE RODENTS TO A SUDDEN, STARTLING NOISE TO SEE IF THEY JUMP. MANY DID: THE ONCE DEAF MICE COULD HEAR.

Getting in, however, was only part of the hearing therapy problem. The other part was identifying the mutations that led to defective hair cells. Researchers started to do this in the 1990s by identifying families with hearing and vision loss typical of Usher and comparing their genomes. These individuals had several genes that seemed to be involved with both ear and eye development, making them prime suspects. Scientists then bred mice both with and without those suspect mutations to test whether any of the genetic changes were truly responsible for symptoms. The comparison pointed a finger at a few specific alterations. Changes to a gene called *USH2A,* for example, are behind gradual disorders such as Corderman's; the nonmutated version of the gene produces healthy hair cells. Each case of the most severe and rapid-onset form of the disease, called Usher type 1, is associated

© 2019 Scientific American

USING A VIRUS to ferry healthy genes into deaf mice, Gwenaëlle Géléoc and Jeffrey Holt, researchers at Boston Children's Hospital, restored a sense of hearing to the rodents.

with mutations in one of five different genes, such as one called *USH1C*.

In the past several years Géléoc's team put all these pieces together. In the lab, she and her husband, otolaryngologist Jeffrey Holt, and others took an AAV version with a customized shell, scooped out a bunch of its genes related to the life cycle of the virus and replaced them with intact, healthy versions of *USH1C*. They also added a DNA sequence called a promoter that turns *USH1C* on in the hair cells. The new gene acted like a booster for the cells when it was delivered. The cells still had the old, faulty DNA that made weak hair proteins, but the addition of well-functioning DNA helped them create a large batch of other healthy proteins that kept the hairs strong.

The team at Boston Children's Hospital then took the entire package and, using a surgical insertion method similar to the one I watched György use, put it inside Usher mice. Within two weeks the virus had infected at least some of the ear hair cells; by six weeks it had penetrated around 80 percent of them. More to the point, these animals reacted to sounds. One of the main tests of restored ear function involves exposing the rodents to a sudden, startling noise to see if they jump. Many did: the once deaf mice could hear.

Sound was not the only important check. Hair cells do another key job in the body: they create a sense of balance and orientation as the hairs flex within the fluid of the inner ear, sending signals about their position to the brain. Mice with hairs damaged by Usher often have difficulties with movement and figuring out where they are in a space. Instead of sniffing around a cage, these rodents cower in the corner. And although mice are natural-born swimmers—ready to thrive the first time they are placed in the water—an Usher mouse will frantically paddle in circles for a few seconds, struggling to determine which side is "up." (Scientists quickly rescue it before the animal becomes too distressed.) If the gene therapy mice at Boston Children's Hospital truly had restored hair cell function, these problems should have gone away.

When I visited Holt and Géléoc's lab right after my trip to Harvard, I saw mice that acted remarkably like, well, mice. Rodents that had gene therapy two months prior nosed around their environment; their behavior in open spaces and in the water was virtually indistinguishable from their normal counterparts. They moved so easily that I repeatedly asked Holt and his team if they were sure these were the mice with mutations. The scientists, who have a careful system for tracking their animals, assured me each time that I was looking at damaged and treated rodents.

© 2019 Scientific American

THE SIZE OF THE PROBLEM

DESPITE THIS SUCCESS, there are still several vexing difficulties to sort out before these viruses are tried on people. One problem is that the current AAVs are too small. Although they are big enough to carry genes to correct Usher type 1C—the disorder that responded so well in mice trials—many other types involve much larger genes. Corderman's form of Usher, for instance, involves a gene that is simply too big to squeeze into the available AAV storage space. "It would be like trying to fit a size-14 body into size-4 pants," Dunbar says.

One work-around would be to slice up the large gene into several chunks that could be carried through the ear in multiple viral vehicles. Each of the gene bits would have sticky ends so that when they arrived at their destination, they could stick themselves back together like Velcro. For example, Corderman's type of Usher gene is so large that it would need to be cut into three parts. For the approach to work, all three virus carriers must get into the inner ear hair cells, and then the three stretches of DNA need to find one another within a cell and paste themselves back together. The highly specific nature of DNA sequences makes this possible—normally in the body, stretches of the genetic alphabet interact only with complementary stretches—but nonetheless difficult to pull off, Géléoc says.

Other options include using a bigger non-AAV virus, tweaked to avoid widespread immune system alarms, or avoiding viruses altogether and trying to deliver the genetic goods within nanoparticles (minuscule lab-made objects that can penetrate cells). Various researchers, including Holt and Géléoc, are also exploring a way to remove the problem gene and replace it with the right one using the gene-editing technique CRISPR-Cas9. Usher is a recessive disorder, caused by two copies of the faulty gene. If researchers could cut out one of these genes and swap in a healthy dominant one, that new gene could take over and swamp the negative effects of the remaining recessive DNA.

No one has yet achieved this feat using CRISPR, however; the method seems better suited to cutting things out than to sticking them in. For that reason, current CRISPR work in mice looks like it would be more effective for rare hearing issues caused by one faulty gene instead of two. The problem gene could simply be knocked out, allowing the remaining gene to do its job properly. The experiments on Beethoven mice used that approach. Although it worked well, other scientists using CRISPR have seen that it caused unwanted DNA changes on other, nontarget cells. For that reason, nobody thinks gene editing of this type is ready for people. Viral vehicles still seem to be the front-runners for therapy.

No matter what delivery system or other solution researchers devise, it will not help many people unless hearing loss diagnoses improve among infants, who would benefit most from early interventions. In the U.S., most newborns are screened for hearing loss, yet they are rarely diagnosed with a specific disorder or its underlying genetic cause. That is exactly what happened to Corderman, who was not diagnosed with a genetic problem until high school. That will need to change so that children can get treated properly.

Gene therapists do think they will be able to treat children—and perhaps sooner rather than later. "It's exciting that there are actual products wending their way through the system," Dunbar says. Elizabeth Olmsted-Davis, a gene therapy researcher at the Baylor College of Medicine, says she is not surprised that gene therapy for other ailments has recently become a clinical reality and that new treatments will eventually follow the same path. "The therapies on the horizon are the culmination of decades of work by talented researchers who saw the potential that these approaches hold," she says.

GENE THERAPY WILL NOT HELP MANY UNLESS HEARING LOSS DIAGNOSES IN INFANTS IMPROVE.

Although interest in gene therapy is clearly surging in the research community, Hannah Corderman is not waiting for the wave to reach her. With or without medical advances, she is determined to live a full life. She has booked multiple trips to watch the Northern Lights in case, one day, she can no longer see them. "I'd say my outlook on life has completely changed because it's like I only have so much time to get things done," she says. She has become an advocate for fellow patients, too. Corderman says she has realized that speaking out about the genetics problems can help push research further and "potentially inspire others to live their lives and not let this disease hold them back." Losing her hearing, she says, does not mean retreating into silence. SA

Dina Fine Maron, formerly an associate editor at *Scientific American,* is now a wildlife trade investigative reporter at *National Geographic.*

MORE TO EXPLORE

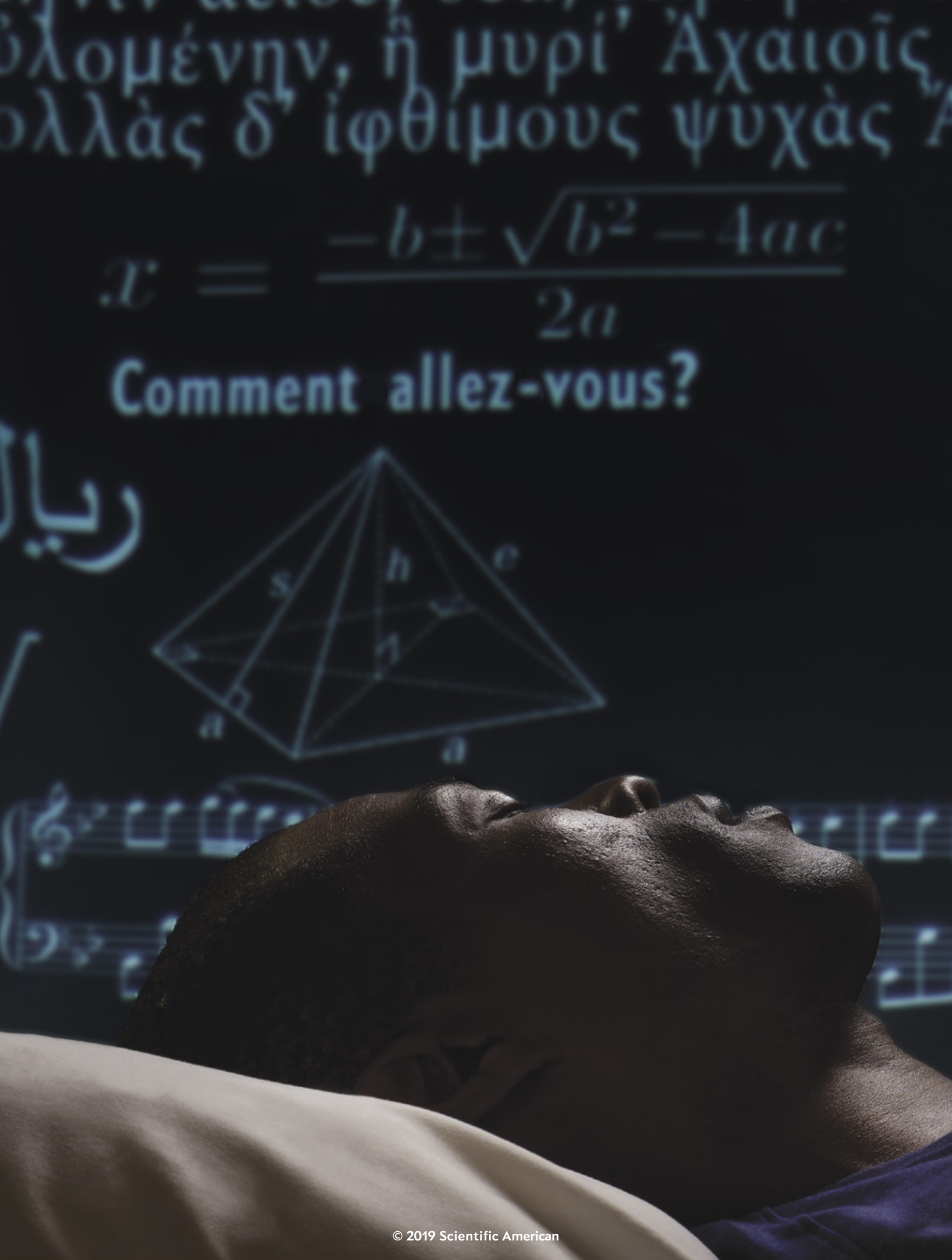
Rescue of Hearing by Gene Delivery to Inner-Ear Hair Cells Using Exosome-Associated AAV. Bence György et al. in *Molecular Therapy,* Vol. 25, No. 2, pages 379–391; February 2017.

Gene Therapy Restores Auditory and Vestibular Function in a Mouse Model of Usher Syndrome Type 1c. Bifeng Pan et al. in *Nature Biotechnology,* Vol. 35, pages 264–272; March 2017.

Cochlear Gene Therapy with Ancestral AAV in Adult Mice: Complete Transduction of Inner Hair Cells without Cochlear Dysfunction. Jun Suzuki et al. in *Scientific Reports,* Vol. 7, Article No. 45524; April 3, 2017.

scientificamerican.com/magazine/sa

© 2019 Scientific American



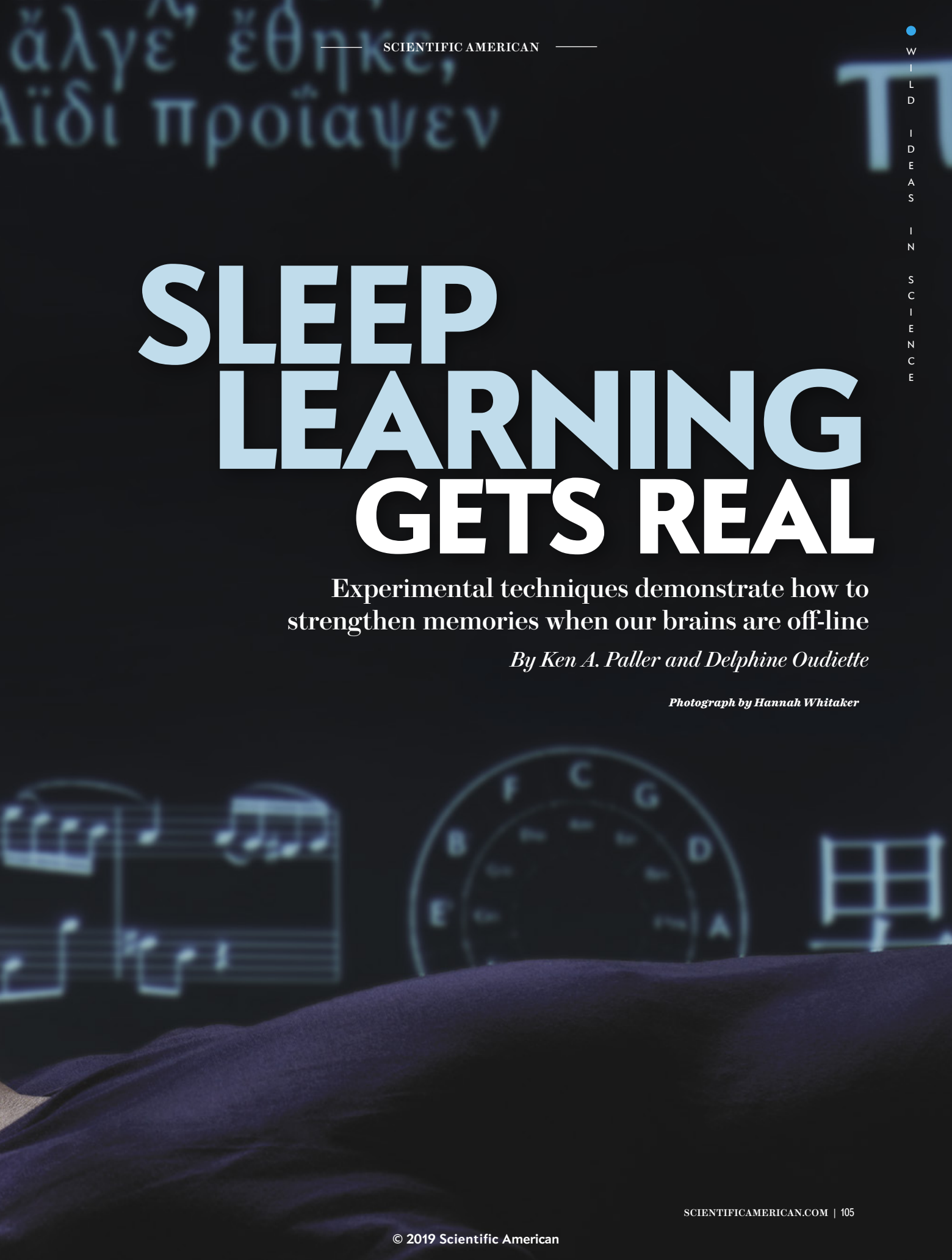
© 2019 Scientific American

SLEEP LEARNING GETS REAL

Experimental techniques demonstrate how to strengthen memories when our brains are off-line

By Ken A. Paller and Delphine Oudiette

Photograph by Hannah Whitaker



© 2019 Scientific American

IN ALDOUS HUXLEY'S *BRAVE NEW WORLD,* A BOY MEMORIZES EACH WORD OF a lecture in English, a language he does not speak. The learning happens as the boy sleeps within earshot of a radio broadcast of the lecture. On awakening, he is able to recite the entire lecture. Based on this discovery, the totalitarian authorities of Huxley's dystopian world adapt the method to shape the unconscious minds of all their citizens.

Sleep learning turns up throughout literature, pop culture and ancient lore. Take Dexter, the lead character in the animated television series *Dexter's Laboratory.* In one episode, Dexter squanders his time for homework, so instead he invents a contraption for learning to speak French overnight. He wakes up the next day unable to speak anything but French. The idea of sleep learning isn't just a modern invention. It also appears within a centuries-old mind-training practice of Tibetan Buddhists; a message whispered during sleep was intended to help a monk recognize the events in his dreams as illusory.

Everyone knows we learn better when we are well rested. Most people, however, dismiss the notion of sleep learning out of hand. Yet a set of new neuroscientific findings complicates this picture by showing that a critical part of learning occurs during sleep: recently formed memories resurface during the night, and this playback can help reinforce them, allowing at least a few to be remembered for a lifetime.

Some studies have even explored whether sleep might be manipulated to enhance learning. They reveal that sleep's program for making daytime memories stronger can be boosted using sounds and odors. Results in rodents have even demonstrated a primitive form of memory implantation: using electrical stimulation while animals slept, researchers taught them where they should go in their enclosures on awakening. Huxley's imagined version of sleep education, in which entire texts are absorbed verbatim during the night, is still relegated to the pages of his 1932 classic. But experiments now indicate that it is possible to tinker with memories while a person is immersed in the depths of slumber, creating the basis for a new science of sleep learning.

THE PSYCHOPHONE

FOR THESE TECHNIQUES to work, scientists have to explore how information can be absorbed when consciousness is seemingly on a well-deserved break. Around the time that Huxley was writing *Brave New World,* serious explorations into the possibility of meddling with sleep had begun. In 1927 New Yorker Alois B. Saliger invented an "Automatic Time-Controlled Suggestion Machine," which he marketed as the "PsychoPhone," to allow a re-

IN BRIEF

Sleep has long remained a mystery, and the possibility of using it to learn has long been disparaged. If the sleeping brain is turned off, the reasoning goes, it cannot learn.

To the contrary, our brains remain highly active during sleep in ways that assist in storing memories. Recent findings, in fact, demonstrate that specific memories are reactivated.

Experimentally controlling the process of memory reactivation makes it possible to study how learning can improve because of nightly periods of downtime.

Future studies that extend this work may examine ways to promote sleep-based problem-solving, eliminate nightmares or perhaps one day gain control over our dreams.

© 2019 Scientific American

corded message to be replayed during the night. The setup seemed to evoke Huxley's imagined technology except that the user, rather than the state, could select the message to be played.

Saliger's invention was followed, in the 1930s and 1940s, by studies documenting ostensible examples of sleep learning. A 1942 paper by Lawrence LeShan, then at the College of William & Mary, detailed an experiment in which the researcher visited a summer camp where many of the boys had the habit of biting their fingernails. In a room where 20 such boys slept, LeShan used a portable phonograph to play a voice repeating the sentence "My fingernails taste terribly bitter." The string of words recurred 300 times each night, beginning 150 minutes after the onset of sleep. The experiment continued for 54 consecutive nights. During the last two weeks of camp, the phonograph broke, so the intrepid LeShan delivered the sentence himself. Eight of the 20 boys stopped biting their nails, whereas none of 20 others who slept without exposure to the recording did so. These early efforts did not use physiological monitoring to verify that the boys were really asleep, though, so the results remain suspect.

The whole field took a severe hit in 1956, when two scientists at RAND Corporation used electroencephalography (EEG) to record brain activity while 96 questions and answers were read to sleeping study participants. (One example: "In what kind of store did Ulysses S. Grant work before the war?" Answer: "A hardware store.") The next day correct answers were recalled only for information presented when sleepers showed signs of awakening. These results led to a shift in the field that persisted for 50 years, as researchers began to lose faith in sleep learning as a viable phenomenon: participants in these experiments appeared to learn only if they were not really sleeping when information was presented to them.

Most scientists during this time tended to avoid the topic of sleep learning, although a few researchers did plug away at asking whether sleep assists in remembering new information. One typical study protocol probed whether overnight sleep deprivation affected recall the day after learning something new. Another asked whether remembering was better after a nap than after an equal period of time spent awake.

Various confounding factors can interfere with such studies. For example, the stress of sleep deprivation can harm cognitive functions that then decrease memory recall. Eventually cognitive neuroscientists began to tackle these challenges by bringing together evidence from multiple research methods. A substantive foundation of evidence gradually accrued to confirm that sleep is a means of reviving memories acquired during the day, reopening the relation between sleep and memory as a legitimate area of scientific study.

Many researchers who took up the challenge focused on rapid eye movement (REM) sleep, the period when dreams are the most frequent and vivid. The guiding assumption held that the brain's nighttime processing of memories would be tied to dreaming, but clear-cut data did not materialize. In 1983 two noted scientists—Graeme Mitchison and Francis Crick, neither psychologists—went so far as to speculate that REM sleep was for forgetting. In a similar vein, Giulio Tononi and Chiara Cirelli, both at the University of Wisconsin–Madison, proposed that sleep could be the time for weakening connections among brain cells, making it easier for new information to be acquired the following day.

Instead of REM, some investigators focused their attention on slow-wave sleep (SWS), a period of deep slumber without rapid eye movements. In 2007 Björn Rasch, then at the University of Lübeck in Germany, and his colleagues prepared people for a sleep experiment by requiring them to learn the locations of a set of objects while simultaneously smelling the odor of a rose. Later, in their beds in the laboratory, sleeping study participants again encountered the same odor as electrical recordings confirmed one sleep stage or another. The odor activated the hippocampus, a brain area critical for learning to navigate one's surroundings and for storing the new knowledge gained. On awakening, participants recalled locations more accurately—but only following cueing from odors that emanated during the course of slow-wave (not REM) sleep.

TARGETED MEMORY REACTIVATION

IN 2009 OUR LAB EXTENDED this methodology by using sounds instead of odors. We found that sounds played during SWS could improve recall for individual objects of our choosing (instead of the recall of an entire collection of objects, as was the case in the odor study). In our procedure—termed targeted memory reactivation, or TMR—we first taught people the locations of 50 objects. They might learn to place a cat at one designated spot on a computer screen and a teakettle at another. At the same time, they would hear a corresponding sound (a meow for the cat, a whistle for the kettle, and so on).

After this learning phase, participants took a nap in a comfortable place in our lab. We monitored EEG recordings from electrodes placed on the head to verify that each individual was soundly asleep. These recordings provided intriguing data on the synchronized activity of networks of neurons in the brain's outer layer, the cerebral cortex, that are relevant for memory reactivation [*see box on next page*]. When we detected slow-wave sleep, we played the meow, whistle and other sounds associated with a subset of the objects from the learning phase. Sounds were presented softly, not much louder than background noise, so the sleeper did not awaken.

On awakening, people remembered locations cued during sleep better than places that had not been flagged during the experiment. Whether sounds or odors served as cues in these experiments, they apparently triggered the reactivation of spatial memories and so reduced forgetting.

At first, the auditory procedures we used were highly controversial. The received wisdom among sleep researchers held that sensory circuits in the cortex are largely switched off during sleep, except for the sense of smell. We were not swayed by this orthodox view. Instead we followed our hunch that the repeated playing of soft sounds might influence the sleeping brain and produce changes in recently stored memories.

Indeed, the same memory benefits were found in many subsequent studies. A technique called functional magnetic resonance imaging highlighted which brain areas take part in TMR, and EEG results brought out the importance of specific brain oscillations. Two papers published in 2018—one by Scott Cairney of the University of York in England and his colleagues; the other by James Antony of Princeton University and his colleagues—linked an oscillation, the sleep spindle, with the memory benefits of TMR.

Besides boosting spatial memory, these procedures have also helped improve recall in other settings. TMR can assist in mastery of playing a keyboard melody and learning new vocabulary

© 2019 Scientific American

The Maestros of Slumber

A complex symphony of neural activity governs the connection between sleep and memory

Brain rhythms provide clues to how sleep helps to store memories for later retrieval. One type of neural signal, called a slow wave, cycling from 0.5 to four times a second, orchestrates the activity of neurons in the cerebral cortex. Each slow oscillation consists of a "down" phase, when neurons are silent, and an "up" phase, when they resume activity. This timing pattern helps to reinforce recently formed memories by ensuring that multiple cortical regions remain in an up state at the same time.

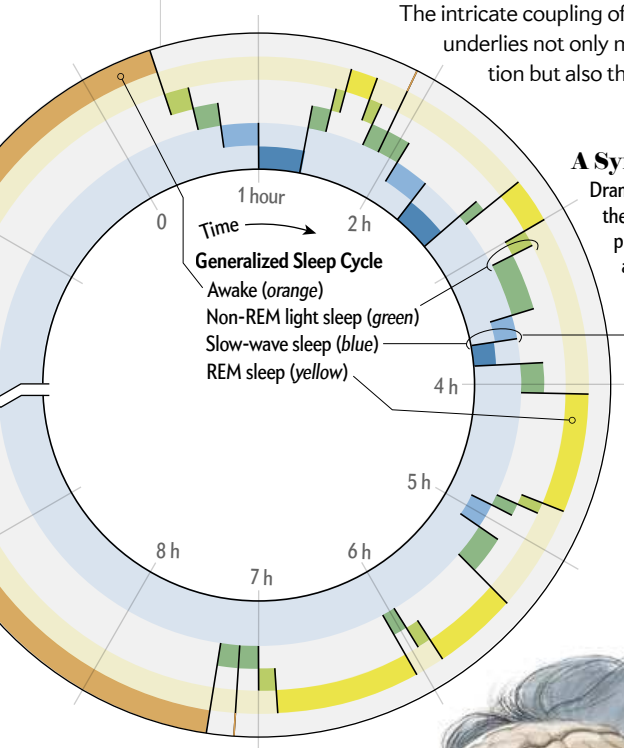
The up phase can coincide with sleep spindles, brief increases of a rhythm of 12 to 15 cycles per second. Spindles originate in the thalamus, which serves as a crossroads for information that is transmitted to virtually all parts of the cerebral cortex. Spindles have a rhythm of their own, recurring at approximately five-second intervals. They coordinate the activity of sharp-wave ripples in the hippocampus. Ripples, for their part, are concurrent with the replay of memories. Slow waves, all the while, assume the role of orchestra conductor: their measured oscillations in the cortex coordinate the pacing for sleep spindles and sharp-wave ripples. The intricate coupling of these oscillations underlies not only memory reactivation but also the altering of connections among neurons to strengthen memory storage. A dialogue between the hippocampus and the cortex involving all these brain rhythms triggers a set of complex network interactions. Through this process, known as consolidation, new information can become integrated with existing memories. The intertwining of memories, moreover, enables the gist of recent experiences to be extracted to make sense of a complex world.

Memory difficulties can arise when this neural dialogue becomes impaired. Individuals with major damage centered in the hippocampus or parts of the thalamus may develop a profound amnesia. Without the expected interactions with these brain regions during both sleep and waking, the cortex cannot store mental records of facts and events known as declarative memories. In addition, a milder form of memory disorder may result when memory processing during sleep is seriously disrupted.

As our understanding of the physiological orchestration of the sleeping brain continues to expand, new strategies may be used to enhance the brain's natural rhythms with various forms of electrical or sensory stimulation. Humans have always had such inclinations, having taken advantage of a lullaby's rhythm or rocking motions to lull a baby to sleep. *—K.A.P. and D.O.*

A Symphony in Two Movements

Dramatic differences characterize two key sleep phases. The slow waves of deep sleep dominate the early part of the night. During slow-wave sleep, some memories spontaneously reactivate. Interventions that promote this process can ensure that memories are retained. Rapid eye movement (REM) sleep prevails in the latter part of a night's slumber, but how it interacts with memory remains controversial.



Harmonizing Brain Waves

Brain oscillations during sleep appear to play a role in strengthening new memories. A key event is the "up" phase of a slow oscillation that coordinates the activity of other brain rhythms. The ascending part of a slow oscillation in the cortex synchronizes with sleep spindles in the thalamus. The spindles coordinate the activity of sharp-wave ripples in the hippocampus. Ripples tend to coincide with a spindle trough.

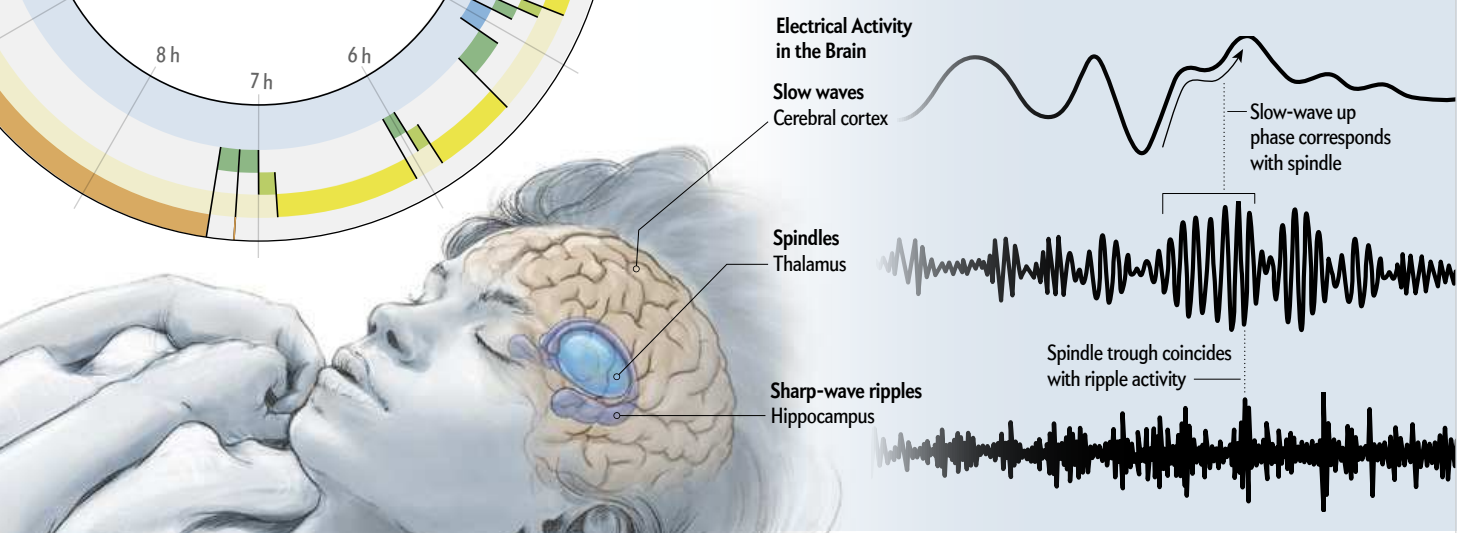


Illustration by Mesa Schumacher

© 2019 Scientific American

or grammatical rules. The technique can also help with simpler types of learning, such as adjustments in one's body image. In conditioning experiments, TMR alters prior learning of an automatic reaction to a stimulus caused by an earlier pairing of that stimulus with an electric shock. Ongoing studies are examining still other types of recall, such as associating names with faces when first meeting new people.

As the technology evolves, TMR should be tested to see if it could help to treat various disorders, reduce addictions or speed recovery from illness. Together with Northwestern University neurologist Marc Slutzky, our lab has been testing a novel rehabilitation procedure for recovering arm-movement abilities after a stroke. Cue sounds are incorporated as part of the therapy and are replayed during sleep to try to accelerate relearning of lost movements. The prospects appear promising because TMR can alter similar forms of motor learning in healthy individuals.

FUTURE PROGRAMS FOR SLEEP LEARNING MIGHT HELP IN PRESERVING MEMORIES, SPEEDING ACQUISITION OF NEW KNOWLEDGE, OR EVEN CHANGING BAD HABITS SUCH AS SMOKING.

WHAT ABOUT LEARNING FRENCH?

THE DEMONSTRATED ABILITY to reinforce memories raises the question of whether new information can be loaded into a person's brain after falling asleep, a technique that calls forth the ethical specter of mind control invoked by *Brave New World*. Is it going too far, though, to imagine that memories can be created surreptitiously?

Although the orthodox response to such conjectures has for many years been an unqualified no, studies by Anat Arzi, now at the University of Cambridge, and her colleagues demonstrated the creation of relatively simple memories using odors. In one experiment, the researchers succeeded in diminishing the desire for tobacco in smokers who were keen to quit. When asleep, study participants were exposed to two odors, cigarette smoke and rotten fish. During the following week, those who had smelled the mix of both odors lit up 30 percent less, having apparently been conditioned to associate smoking with the aversive fish odor.

Acquiring a more complex memory is not as easy, but even that may one day prove possible. Karim Benchenane of the French National Center for Scientific Research (CNRS) and his colleagues have shown how to literally change the mind—of a mouse. When they began their work, Benchenane and his team knew that when a mouse explores a new environment, neurons called place cells fire as the animal traverses specific parts of an enclosure. These same neurons discharge again during sleep as the memory is apparently replayed.

The researchers stimulated the reward system of the mouse brain (the medial forebrain bundle) precisely when place cells became spontaneously active while the animal was asleep. Amazingly, mice subsequently spent more time at the locations that corresponded to the stimulated place cells, heading there directly after they woke up. More experiments still need to disentangle whether fully formed false memories were implanted in the mice during sleep or whether they were automatically guided to those spots by a process of conditioning, without any knowledge about why they were drawn to those locations.

The boundaries of what may be possible remain to be tested, but this research has established that a normal component of learning continues nocturnally off-line. Sleep is needed not just to stay alert and rejuvenated but also to reinforce memories initially acquired while awake. We still need to learn much more about off-line memory processing. Further work must ascertain how sleep helps learning and which brain mechanisms are engaged to preserve the most valuable memories. It is also essential to find out more about the perils of poor or inadequate sleep that might be affected by various forms of life stress, certain diseases or the experience of growing older.

A study led by Carmen Westerberg, then at Northwestern, points in the desired direction. Westerberg tested patients with the memory dysfunction that often precedes Alzheimer's disease—amnestic mild cognitive impairment. The results documented a link between poor sleep and reduced ability to remember information after an intervening overnight delay.

All of this knowledge might help in creating programs of sleep learning to preserve memories, to speed the acquisition of new knowledge, or even to change bad habits such as smoking. Looking still further ahead, scientists might also explore whether we can gain control over our dreams, which could lead to the prospect of nightmare therapies, sleep-based problem-solving and perhaps even recreational dream travel. In a culture that already offers wrist-based sleep trackers and mail-order gene tests, we can begin to contemplate new ways to convert daily downtime into a productive endeavor—for some, a chilling prospect, and for others, another welcome opportunity for hacking the self. SA

Ken A. Paller is a professor of psychology and director of the cognitive neuroscience program at Northwestern University. His recent research on targeted memory reactivation was funded by the U.S. National Science Foundation.

Delphine Oudiette is a research associate for the French National Institute for Health and Medical Research (INSERM) at the Brain & Spine Institute and at the sleep disorder unit located at Pitié-Salpêtrière Hospital, both in Paris.

MORE TO EXPLORE

The Secret World of Sleep: The Surprising Science of the Mind at Rest. Penelope A. Lewis. Palgrave Macmillan, 2013.

Upgrading the Sleeping Brain with Targeted Memory Reactivation. Delphine Oudiette and Ken A. Paller in *Trends in Cognitive Sciences,* Vol. 13, No. 3, pages 142–149; March 2013.

Why We Sleep: Unlocking the Power of Sleep and Dreams. Matthew Walker. Scribner, 2017.

Sleeping in a Brave New World: Opportunities for Improving Learning and Clinical Outcomes through Targeted Memory Reactivation. Ken A. Paller in *Current Directions in Psychological Science,* Vol. 26, No. 6, pages 532–537; December 2017.

scientificamerican.com/magazine/sa

© 2019 Scientific American

SPIKY PROTEIN COAT of the rabies virus (*shown magnified roughly a million times*) enables it to pass from neuron to neuron.

© 2019 Scientific American

RABIES ON THE BRAIN

Using engineered forms of the rabies virus, neuroscientists can map brain circuits with unprecedented precision

By Andrew J. Murray

© 2019 Scientific American

LATE ONE MOONLIT NIGHT, three fictional revelers on an English moor were transfixed by a horrific sight: "a foul thing, a great, black beast, shaped like a hound, yet larger than any hound that ever mortal eye has rested upon. And even as they looked the thing tore the throat out of Hugo Baskerville, on which, as it turned its eyes and dripping jaws upon them, the three shrieked with fear and rode for dear life." Historians of medicine have traced the terror that the *The Hound of the Baskervilles* evoked in Arthur Conan Doyle's fans to the profound impact of rabies on contemporary British consciousness. With an ability to turn the most placid of pets into frothing, raging beasts and an almost 100 percent mortality rate, the rabies virus was one of the most feared scourges in human history.

As early as 1804, experiments by German physician Georg Gottfried Zinke indicated that the virus occurs in high concentrations in the saliva of an infected animal. The germ also acts to enhance the production of saliva while increasing the amount of it present in the mouth—explaining why rabid dogs drool. Louis Pasteur went on to demonstrate in the 1880s that the brain, too, is infested with the virus. None of this is an accident. Two centuries of research have now established that the rabies virus combines a propensity to be transferred from the saliva-soaked jaws of an infected animal with a diabolical ability to drive it into a frenzy of aggressive biting. By a feat of evolution, the virus manipulates the host's brain to ensure its own efficient transmission.

Rabies still kills more than 59,000 people annually. Thanks to vaccinations and the quarantine of infected animals, however, it no longer evokes terror in the developed world. Rather neuroscientists are turning the malign germ to the advantage of humankind. The rabies virus is adept at making its way from the site of the bite to the brain by jumping stealthily from neuron to neuron—thereby evading detection by the immune system. A number of researchers, including those in my group at the Sainsbury Wellcome Center for Neural Circuits and Behavior in London, have now harnessed and refined this ability to visualize the connections between neurons.

The human brain consists of billions of neurons, each connected to thousands of others; mapping this tangled web of wires is essential for understanding how it generates our emotions and behaviors. Using engineered varieties of the rabies virus, we can now observe what kinds of inputs a particular type of neuron receives, how electrical signals move from the eye to the brain and what types of neurons control posture to keep us from falling over. Although the field is still in its infancy, in the future such information could help us understand, and perhaps find remedies for, neurological disorders such as Parkinson's disease.

FROM BITE TO BRAIN

TO BEGIN WITH, the bite injects virions, or virus particles, into muscle tissue. A bullet-shaped capsule containing a single strand of RNA and proteins, the rabies virion is coated with a spiky protein, called a glycoprotein. This coat tricks motor neurons that send projections to the assault site into bringing the virus inside. Motor neurons emit chemicals that cause muscles to contract, and they are linked by a long chain of other neurons to the victim's brain—the virus's ultimate destination.

To be precise, the glycoprotein binds to a receptor on a synaptic terminal of the neuron: a point where it transmits signals to a neighboring neuron. Like a door through which one only exits the secure area of an airport—but not enters—the synaptic terminal guards a one-way passage—a synapse—between the neurons. By convention, the "downstream" direction of the synapse is the flow of signals from one neuron to the next, all the way from the brain to the muscles. The rabies virus travels upstream, however, because it has to get to the brain. As such, it fools the receptor to enter a motor neuron through the exit gate.

Viruses are adept at using their host's cells for their own purposes, but few can beat rabies at the task. Once inside, the intruder throws off its glycoprotein disguise, and its RNA gets to work, using the cell's materials and metabolism to produce copies of itself, as well as of all its characteristic proteins. These components then reassemble to create daughter virions. Whereas many

IN BRIEF

The rabies virus is adapted to jumping from one neuron to another as it makes its way from the site of a bite to an animal's brain.

Virologists and neuroscientists have harnessed this capability to identify the neurons that send signals to the particular neurons they are studying.

The technology involves engineering the rabies virus so that it glows, infects only the neurons of interest and can jump once across a connection.

PAGE 110: CHRIS BJORNBERG *Science Source*

© 2019 Scientific American

virus species replicate so rapidly that they force the infected cell to burst open, releasing the virions into the space between the cells, the rabies virus strictly regulates its reproduction—producing just enough daughters to keep moving on. That way, it refrains from causing so much damage that it alerts the immune system. Instead it leaves the host cell intact and crosses a synapse to a new upstream neuron. That sneakiness is one reason the disease has such a long, symptomless incubation period, typically one to three months in humans.

Having thus jumped to a new neuron, the virion starts the entire process again: undressing and copying itself and reassembling daughters that move into the next upstream neuron. In this way, the rabies virus picks a path through the nervous system, creeping from the motor neuron it first encountered in the muscle tissue, through the spinal cord and into the brain.

By the early 2000s several research groups, including those of Gabriella Ugolini, now at the Paris-Saclay Institute of Neuroscience, and Peter Strick, now at the University of Pittsburgh, were pursuing the use of rabies as a tracer for neuronal circuits. Deciphering the route that the virus took from the muscle to the brain was a challenge, however. As a neuroscientist looking at a snapshot of neurons that had been infected with the virus, how could you distinguish between the first jump of the invader from one neuron to the next, the second jump, and so on?

The researchers initially solved this problem by euthanizing laboratory animals shortly after infection, thereby allowing the virus to spread across only one or two synapses. This approach uncovered some of the major pathways in the brain that contribute to motor control. But it had its drawbacks. Not all connections between neurons are equal. A synapse may be strong (or weak), making it more (or less) likely that a signal moving across it will prompt the target neuron to fire in response. Another might be located close to the cell body instead of far away at the end of a projection. And some neurons make a single link with a downstream neuron, whereas others may make hundreds. This heterogeneity means that the virus takes varying lengths of time to travel from one neuron to the next, adding a layer of uncertainty. What if the virus moves through two or three strong synapses before it passes through a weak one?

VIRAL ENGINEERING

TO GET AROUND this problem, scientists needed to rejigger the rabies virus. Molecular biologists have developed the amazing ability to manipulate DNA: swapping out genes has become as routine for them as making coffee in the lab kitchen. The wild rabies virus has no DNA to manipulate, however, only RNA. The advent of reverse genetics, which flips the normal genetic cycle by making RNA from DNA, got around that hurdle. In 1994 Matthias Schnell and Karl-Klaus Conzelmann, both then at the Federal Research Center for Virus Diseases of Animals in Tübingen, Germany, produced a functional rabies virus in the lab from cloned DNA alone. They even altered the rabies genome: the RNA string that encodes its characteristic properties.

The ability to manipulate the genome swiftly led to a greater understanding of how the different rabies genes contribute to the virus's diverse skills. Only one gene was essential to its ability to move between neurons, it turned out: the one that coded for glycoprotein. A rabies virus that had the glycoprotein gene removed from its genome could infect a cell, but once inside it was stuck there. This would be the discovery that thrust the virus into mainstream neuroscience.

In 2007 a collaboration between neuroscientists Ian Wickersham and Edward Callaway, both then at the Salk Institute for Biological Studies in La Jolla, Calif., and virologists Conzelmann and Stefan Finke of the Friedrich Loeffler Institute in Germany resulted in an ingenious system to map neuronal circuits. The first step in their scheme was to swap the glycoprotein gene in the rabies genome with one that coded for a fluorescent protein. The engineered virion could not manufacture glycoproteins; instead its RNA made copies of the fluorescent protein (along with all the other rabies proteins)—so the infected cell shone with a bright color of the experimenters' choosing.

The second step was to provide glycoprotein in the targeted neuron via some other genetic mechanism. That way, the daughter virions could don glycoprotein coats and jump once—but no more. To that end, the scientists harnessed a very simple type of virus, called an adeno-associated virus (AAV) because it is often found along with much larger viruses called adenoviruses. AAVs contain a tiny amount of DNA. The Salk researchers inserted a gene for making the rabies glycoprotein into that DNA. The rabies virion could harness the glycoprotein the gene manufactured to jump across a single synapse. It could not, however, take the glycoprotein gene with it because it was a segment of DNA, not of RNA. So when the virion had jumped into the next cell, it was stuck again. At that point, a glance at the infected animal's brain revealed populations of glowing cells across the nervous system that were directly connected to any neuron researchers wanted to target.

There remained one problem, however. Injection of the rabies virus into the brain resulted in the direct infection of any neuron that sent a projection into the injection site. Without a way to restrict the initial infection of the rabies virus to particular neurons, scientists could not differentiate between neurons that were infected directly by the injected virus and those that were infected after the virus had moved across a synapse. The solution would come from another area of virology: viruses that specifically affect birds.

In the wild, entire classes of viruses can be found that infect only certain groups of animals. For example, the avian sarcoma leukosis virus (ASLV) usually leads to cancer in chickens but cannot normally infect mammalian cells. Like rabies, this virus has a glycoprotein envelope, which comes in a variety of configurations. Different ASLV glycoproteins are known as Env (for envelope), followed by a label for the particular form. Each subtype binds to a specific receptor. So, for example, EnvA binds to a receptor called TVA (for avian *t*umor receptor *v*irus *A*). If a cell does not possess the TVA receptor, it cannot be infected with an EnvA-coated virus. This selective interaction enables researchers to restrict the initial infection of rabies virus to one type of neuron.

By introducing the gene for EnvA glycoprotein in a rabies-infested cell culture (a process known as pseudotyping), Wickersham, Callaway and their colleagues replaced the native glycoprotein coat on the rabies virus with the EnvA glycoprotein from the avian virus. Thus altered, the rabies virus could not deceive any mammalian cells into letting it in. By endowing the neuron of interest, typically in a mouse brain, with the TVA receptor, neuroscientists could be assured that the rabies virus would infect only this cell.

The target neuron (in practice, a class of neurons) was also supplied with an AAV containing the gene for rabies glycoprotein. Once inside, the rabies virus shed its chicken costume,

© 2019 Scientific American

Using Rabies to Track Brain Circuits

The rabies virus makes its way from the bite to the brain by jumping from one neuron to the next. Virologists and neuroscientists have harnessed and modified this ability to see how neurons connect into complex circuits.

NORMAL RABIES PATHOLOGY

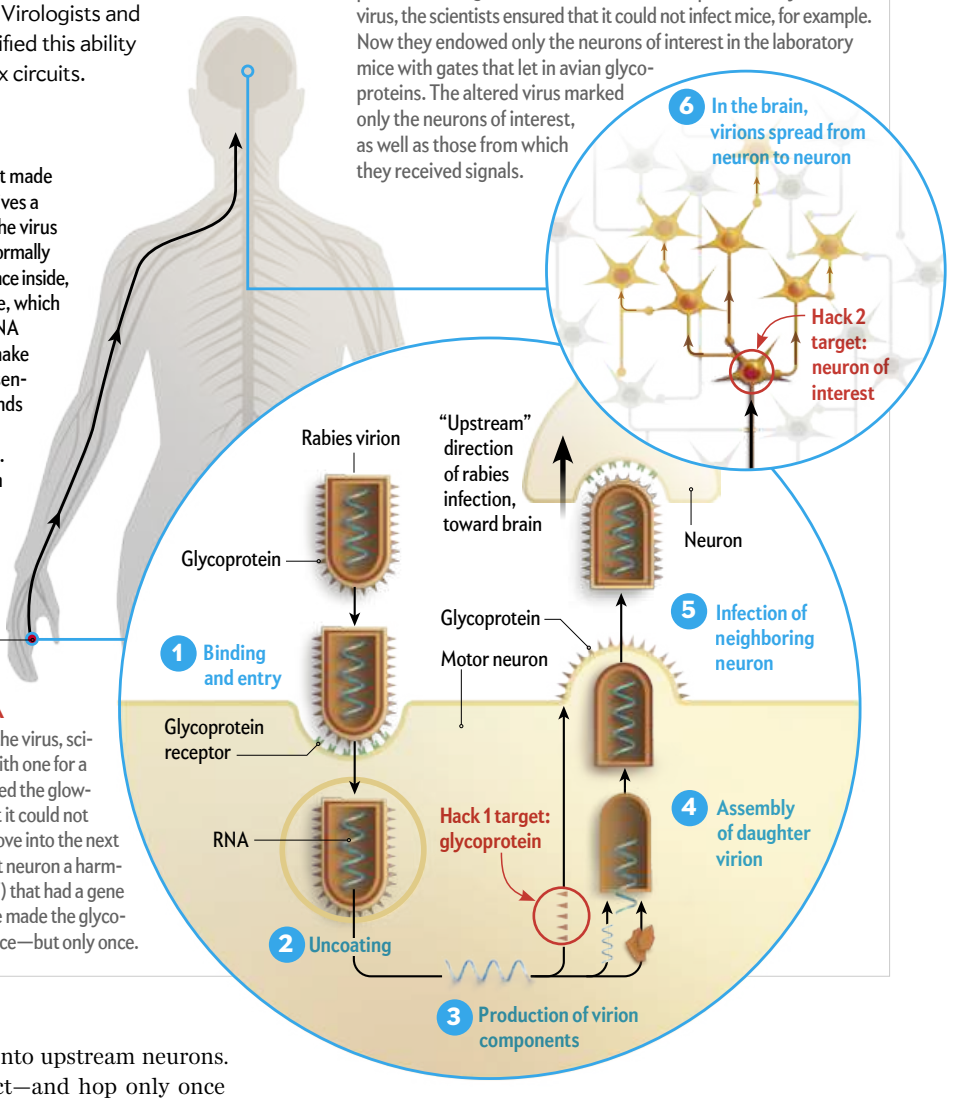
The rabies virion, or virus particle, has a coat made of glycoprotein (a type of protein) that deceives a nearby motor neuron into letting it in 1. The virus enters at a synaptic terminal, or gate, that is normally used to send information to other neurons. Once inside, the virus sheds its coat to release its genome, which is made of RNA rather than DNA 2. The RNA uses the neuron's metabolic machinery to make multiple copies of itself and of the virus's essential proteins 3. The proteins and RNA strands reassemble into daughter virions 4 that move upstream into connected neurons 5. In this manner, the virus moves from neuron to neuron on its way to the brain, where it continues to propagate 6.

HACK 1: ENGINEERING RABIES RNA

To ensure that they could follow the exact route of the virus, scientists replaced the glycoprotein gene in its RNA with one for a fluorescent protein. The modified RNA manufactured the glowing protein, so that the infected neuron glowed, but it could not make the glycoprotein. Thus, the virus could not move into the next neuron. Next, the researchers added into the target neuron a harmless virus (called an adeno-associated virus, or AAV) that had a gene for rabies glycoprotein added to its DNA. That gene made the glycoprotein, which the virions could harness to jump once—but only once.

HACK 2: ALTERING THE GLYCOPROTEIN COAT

To restrict the rabies infection to the neurons of interest, experimenters used the fact that glycoprotein comes in various types. The glycoprotein coats worn by certain viruses that target birds cannot normally enter mammalian cells. So by replacing the usual glycoprotein covering of the rabies virus with that possessed by an avian virus, the scientists ensured that it could not infect mice, for example. Now they endowed only the neurons of interest in the laboratory mice with gates that let in avian glycoproteins. The altered virus marked only the neurons of interest, as well as those from which they received signals.



picked up its normal cloak and jumped into upstream neurons. By engineering the rabies virus to infect—and hop only once from—a well-defined group of "starter" neurons, researchers could now get a clear image of how the brain was wired.

TUNING RABIES

THE SIMPLICITY AND ELEGANCE of the delta-G rabies system (as its inventors called it because of the altered glycoprotein) took the neuroscience community by storm. Using it, researchers could see right away what kinds of neurons send signals to the neurons of interest. Like all new technologies, however, the scheme had its imperfections. Sometimes the number of connections labeled were rather small—on the order of 10 per starter neuron.

Around 2015 Thomas Reardon, Thomas Jessell, Attila Losonczy and I, all then at Columbia University, were using the delta-G system to understand the neural circuits that guided motor commands. Finding relatively low numbers of connections to motor neurons in the spinal cord or the brain, we suspected we were getting an incomplete picture of the circuitry. Another issue was neurotoxicity. Once the virus was in a cell, it would start to break down and die within a couple of weeks. If the virus itself was causing individual neurons to alter their behavior, interpreting any observations could be problematic.

Schnell and Christoph Wirblich, both at Thomas Jefferson University, had done pioneering work on rabies virus biology, so we went to them for help. They knew right away that our problems stemmed from the strain of virus that we were using. It had originally been developed for use in a rabies vaccine. Vaccines incorporate special strains of the germ that humans have selected to reproduce unusually rapidly so that the multitudinous daughter virions explode out of the infected cells and alert the immune system before it is too late. That indicated a way to refine our research tool. Because we were using mice in our studies, our

Illustration by Kelly Murphy

© 2019 Scientific American

virologist collaborators suggested that we instead try a strain that had been tuned over many years to infect mouse neurons.

The parent virus of this strain had originally been isolated in the wild and then "fixed" in the lab by being repeatedly passed through the brains of mice or through cell lines. It had thereby evolved to be a specialist at targeting the mouse nervous system. After assembling a neuronal tracing mechanism based on this mouse-specific strain, we found that it labeled many more connections than we had previously seen. Moreover, being an expert at evading the mouse immune system, it made relatively small amounts of each protein. As such, it placed less strain on the host cell's machinery and allowed neurons to remain relatively healthy.

We further altered our tracing system to replace the gene for the fluorescent protein in the rabies virus with one for a light-sensitive protein, called channelrhodopsin (ChR), originally found in green algae. When activated by blue light, this remarkable molecule opened a channel that allowed positively charged ions to flow into the target neuron, prompting it to emit an electrical signal. (The infected cell continued to glow, however, because we used a version of ChR that included a fluorescent protein.) With this fine-tuned rabies virus system, we could watch entire neuronal circuits fire during certain actions of the mouse or switch them on or off—for up to a month after the virus had infected a neuron. That gave us ample time to conduct many of the tests we needed to understand how specific circuits generate behavior.

WIRING DIAGRAM

USING DIFFERENT VERSIONS of the delta-G rabies system, neuroscientists have probed many different circuits in the nervous system to understand how they contribute to the perceptions and behaviors of animals. Take, for instance, the visual system. When light enters the eye, neurons at the back of the retina, called retinal ganglion cells, transmit signals to the brain. Neuroscientists traditionally believed that this information travels to intermediate locations in the brain, ultimately ending up in the cerebral cortex—the celebrated gray matter—where it is processed. Botond Roska's group at the Friedrich Miescher Institute for Biomedical Research in Switzerland used the rabies system to trace the inputs from the retinal ganglion cells to the lateral geniculate nucleus (LGN), an area of the brain that was regarded as just another relay to the cortex.

The researchers demonstrated that the LGN contained three different types of neurons, each likely processing visual information differently. Indeed, less than a third of the neurons served as a relay, providing a direct line from the retina to the cortex. But roughly another third received combinations of different inputs from one eye; the remaining neurons (about 40 percent) got signals from both eyes. Thus, although the LGN lies at an early stage of the visual circuit, most of its neurons integrate information from multiple sources. The finding will likely illuminate the process by which the brain interprets information from the eyes.

At Columbia, my co-workers and I investigated the neurons in the lateral vestibular nucleus (LVN), a brain region that tries to prevent us from falling over. Imagine being on a moving subway train that stops unexpectedly. Before you have had time to think, you shift your feet to compensate, stiffen your legs and perhaps grab the nearest pole. How does the brain activate the right groups of muscles so swiftly in a variety of similar situations?

We found that the LVN of mice contains two anatomically distinct types of neurons, each having different downstream connections to parts of the nervous system. One group switches on very quickly after your brain senses your body is unstable; these neurons act to extend the limbs to widen the base of support. Later, a second set of LVN neurons become active. These serve to strengthen and stabilize the joints in the same limb, enabling the body to be pushed back to its original position. We could activate these neurons simply by switching on a blue light, delivered to the LVN by a fiber-optic cable. When the light came on, the mice adjusted the positions of their limbs, as if to stop themselves from falling over—even when they were not off-balance.

Nao Uchida's lab at Harvard University investigated a third significant question: What are the functions of neurons that release dopamine? Such "dopaminergic" neurons in two regions of the brain, the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), have long been known to respond to rewards. They would become very excited when a test animal got a treat or when a sensory stimulus predicted that it was about to come. (Think of eating a candy bar, compared with hearing the rustling of its wrapper.) To understand what types of information the neurons were receiving, scientists needed to know how they were connected to other brain circuits. Using the delta-G system, the Harvard team found that dopaminergic neurons in the SNc received information about the relevance of a stimulus: Is this sound of a candy wrapper going to get me a piece of chocolate? In contrast, the VTA received information on the quality of the reward: How good is this candy?

As it happens, dopaminergic neurons in the SNc degenerate in Parkinson's. Intriguingly, Uchida and his colleagues also discovered that major inputs into such neurons in the SNc come from the subthalamic nucleus, a small, lens-shaped region of the brain that, along with similar nuclei, is involved in controlling movement. Exciting the subthalamic nucleus by means of an inserted electrode, in a technique known as deep-brain stimulation, is often effective at relieving symptoms of Parkinson's. Surmising that the inputs they had discovered explained why such stimulation works, the neuroscientists reasoned that targeting other brain regions, which they had identified as also sending inputs to the SNc, might aid some Parkinson's patients.

The combination of natural evolution and targeted engineering has thus given neuroscientists a remarkably powerful tool. There is still much room for improvement. For example, will it be possible to engineer viruses that move downstream, labeling a neuron's outputs instead of its inputs? Can we make a virus that labels only active connections between neurons, lighting up the circuits that are involved in distinct behaviors? The time has come for a virus that has manipulated and terrorized humans for millennia to be manipulated to serve us. SA

Andrew J. Murray is a neuroscientist at the Sainsbury Wellcome Center for Neural Circuits and Behavior in London. His group studies how brain circuits generate movement.

MORE TO EXPLORE

Monosynaptic Restriction of Transsynaptic Tracing from Single, Genetically Targeted Neurons. Ian R. Wickersham et al. in *Neuron,* Vol. 53, No. 5, pages 639–647; March 1, 2007.

Whole-Brain Mapping of Direct Inputs to Midbrain Dopamine Neurons. Mitsuko Watabe-Uchida et al. in *Neuron,* Vol. 74, No. 5, pages 858–873; June 7, 2012.

scientificamerican.com/magazine/sa

© 2019 Scientific American

Googling Heroin

Internet searches offer a novel way to predict overdose deaths • By Rod McCullom

ABOUT 115 PEOPLE NATIONWIDE DIE EVERY DAY FROM opioid overdoses, according to the U.S. Centers for Disease Control and Prevention. A lack of timely, granular data exacerbates the crisis; one study showed opioid deaths were undercounted by as many as 70,000 between 1999 and 2015, making it difficult for governments to respond. But now Internet searches have emerged as a data source to predict overdose clusters in cities or even specific neighborhoods—information that could aid local interventions that save lives.

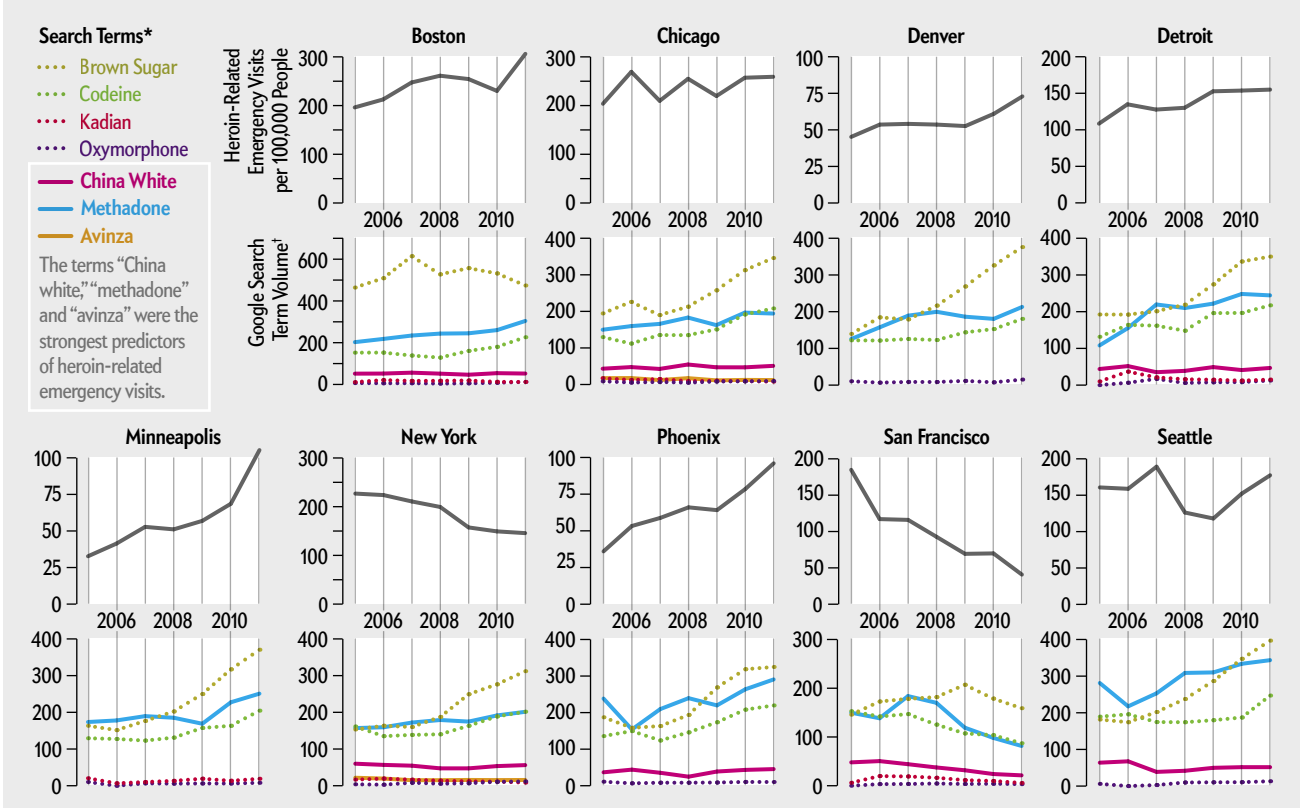
The working hypothesis was that some people searching for information on heroin and other opioids might overdose in the near future. To test this, a researcher at the University of California Institute for Prediction Technology (UCIPT) and his colleagues developed several statistical models to forecast overdoses based on opioid-related keywords, metropolitan income inequality and total number of emergency room visits. They discovered regional differences (*graphic*) in where and how people searched for such information and found that more overdoses were associated with a greater number of searches per keyword. The best-fitting model, the researchers say, explained about 72 percent of the relation between the most popular search terms and heroin-related E.R. visits. The authors say their study, published in the September 2018 issue of *Drug and Alcohol Dependence,* is the first report of using Google searches in this way.

To develop their models, the investigators obtained search data for 12 prescription and nonprescription opioids between 2005 and 2011 in nine U.S. metropolitan areas. They compared these with Substance Abuse and Mental Health Services Administration records of heroin-related E.R. admissions during the same period. The models can be modified to predict overdoses of other opioids or narrow searches to specific zip codes, says lead study author Sean D. Young, a behavioral psychologist and UCIPT executive director. That could provide early warnings of overdose clusters and help to decide where to distribute the overdose reversal medication Naloxone.

Still, this approach has limitations. Not everyone uses Google, and some search terms lacked important context: "brown sugar" (slang for a type of heroin) was the most popular one for opioids in the majority of cities studied, but the researchers noted that their model could not distinguish it from the baking ingredient. In addition, the overdose data in the study were relatively old.

Jeanine Buchanich, a biostatistician at the University of Pittsburgh, who was not involved in the prediction study, says that "the paper highlights the need for new, innovative approaches to analyzing data related to the opioid epidemic." SA

Rod McCullom is a science writer whose work has appeared in *Nature, Scientific American,* the *Atlantic, The Nation* and Undark Magazine, among others.



*Search terms may refer to slang for heroin and other opioids.
†Google search term volume is the probability of the search based on geography and time period, multiplied by 10 million for readability.

SOURCE: "INTERNET SEARCHES FOR OPIOIDS PREDICT FUTURE EMERGENCY DEPARTMENT HEROIN ADMISSIONS," BY SEAN D. YOUNG ET AL., IN *DRUG AND ALCOHOL DEPENDENCE*, VOL. 190; SEPTEMBER 1, 2018

Graphic by Amanda Montañez

© 2019 Scientific American

Advertisement

CRUISE WITH SCIENTIFIC AMERICAN Travel

The vistas, the music and museums, the food and the whisky — all memorable. But it's the people of the British Isles who give the sense of place you'll bring home. From Inverness to Liverpool, Northern Ireland to the Republic of Ireland, experience the region's common cultural elements and distinctive local ways of life with Scientific American.

While at sea, enjoy extensive seminars and exclusive receptions with leading scientists passionate about sharing their work in astronomy, psychology and more. In port, survey the terrain from the elemental to urbane. Join Scientific American in an adventure balancing an illuminating science program and group activity with plenty of time to relax and experience the British Isles.

GREAT BRITAIN, AUGUST 13th – 25th, 2019

FOOD AND DINING

Dining on board a cruise ship reflects the passion, global perspective, and expertise the culinary staff brings to providing a personalized, relaxing, vacation meal experience for you.

Cruise prices start at $2,809 per person (pp) based on double occupancy. For those attending our SEMINARS, there is a $1,575 fee. Add'l pp fees, taxes, and gratuities apply. The Program, cruise pricing, and options are subject to change.

For more info. call 650-787-5665, visit InsightCruises.com, or email info@InsightCruises.com

SEMINARS

The conference fee is $1,575 and includes all 90-minute seminars below.

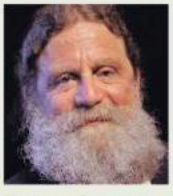
MEDICINE:

- State of the Art in Medicine
  - — Bones
  - — Joints
  - — Spine
- How Smart Orthopaedic Implants Work

Eric Ledet, Ph.D.

PSYCHOLOGY:

- Stress, Coping, and Health
- Biology and Psychology of Depression
- The Biology of Human Behavior at Our Best and Worst
- Biology of Schizophrenia
- Biology of Aggression and Violence



Robert Sapolsky, Ph.D.

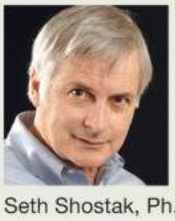
ENGINEERING:

- Carrickfergus Castle: Medieval Military Aspects
- The Walls of Londonderry: Unbreached
- Great Britain & Ireland's Great Bridges
- Firth of Forth: Now *That's* a Cantilever Bridge

Stephen Ressler, Ph.D.

ASTRONOMY:

- How the 21st Century Will Change Everything
- Looking for Life Beyond Earth
- What Would the Real Aliens be Like?
- Could the Aliens be Here?
- The Future of Everything



Seth Shostak, Ph.D.

For speakers' complete bios, visit http://InsightCruises.com/events/sa38/#SPEAKERS.html

Subscribe to our Collector's Editions!

Take Five Deep Dives a Year into Today's Most Exciting Science

A subscription includes five print issues per year & digital access to these issues online. Immerse yourself in riveting tales of research & discovery at the cutting edge of human knowledge in each issue.

SPECIAL COLLECTOR'S EDITION
SCIENTIFIC AMERICAN
MIND
Be a Better You
Smart. Happy. Relaxed
SPECIAL COLLECTOR'S EDITION
SCIENTIFIC AMERICAN
REVOLUTION IN SCIENCE
3 UNSOLVED SCIENTIFIC MYSTERIES
13 DISCOVERIES THAT COULD CHANGE EVERY
SPECIAL COLLECTOR'S EDITION
SCIENTIFIC AMERICAN
DO WE LIVE IN AN INFINITE MULTIVERSE?
WONDERS OF THE COSMOS
Far-out tales from the edges of space and time
Black hole at the beginning of time
Searching for life on other worlds
Why do stars explode?
The largest structures in the universe

sciam.com/collectors-subscription

Scientific American is a registered trademark of Springer Nature America, Inc.